The impacts of translocation on the cultural evolution of song in the North Island saddleback or tīeke (*Philesturnus rufusater*)

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Ka mahi ahau i te whare o te tīeke

(I am making a saddlebacks nest)

Photo: Martin Sanders

Ka whakarongo ake au

I te tangi a tōna waituitui

E rere runga rawa e

tui tui tuituia

Tuiā i runga

Tuiā i raro

Tuiā i roto

Tuiā i waho

Tui tui tuituia

Kua tū

Kua tau

Kua oti

Te whare tā tīeke

Tihei mauri ora

(*I am made peaceful*

by the harmonics of his binding song

Resonating up on high

Weaving, binding, stitching

Uniting what is above

with what is below

Connecting the internal

with the external

Weaving, Binding, stitching

It has been Erected,

It has been Bestowed,

It has been made complete

The sacred home of Tīeke

Indeed it is the vital essence of life abundant issuing forth)

Poem and translation by Zack Bishara

Abstract

The IUCN (1987) defines a translocation as a release of animals with the intention of establishing, re-establishing, or augmenting an existing population. The origins of translocation practise are very much in applied conservation management. However, translocations also provide other outputs. They provide a means by which the general public might connect and commit to conservation and they provide unique opportunities for scientific research because the age and source of founder populations are completely known. Geographical isolation plays a crucial role in speciation events. Thus studies of divergence of behavioural signals in isolated populations have been critical to understanding how barriers to gene flow develop. Bird song is a vital conspecific recognition signal (CRS) and many studies have demonstrated significant geographical variation in song with several hypotheses posed to explain this variation. However, a key problem in testing these hypotheses is an inability to measure the pace of song divergence. This is because the timing and source of founder events are rarely detected. Here I use the NI saddleback or tīeke (*Philesturnus rufusater*) isolated on a single island in 1964 but subsequently increased by translocation to 13 island populations, to show that significant geographical variation in song can develop in less than 50 years. Furthermore, my data shows a clear signal of serial population bottlenecks (up to 3 times) following translocation and supports both bottleneck and cultural mutation hypotheses in explaining this variation. Critically NI saddleback discriminate between songs from different islands and this discrimination might lead to an eventual reduction in effective population size. This illustrates the potential for human induced founder and isolating events, including conservation management, to be microevolutionary events and challenges us to consider the implications of conservation biology in an evolutionary context.

Preface

There is considerable geographical variation in communication signals between many populations of animals including humans and other primates, cetaceans, insects and amphibians. However, this variation is most evident in birds (Podos & Warren, 2007), particularly amongst the passerines which comprise almost half of all bird species (Fitzpatrick, 1988). Thus, geographical variation in bird song has attracted enormous research effort in documenting where it exists, how it might develop and why it is biologically significant (Podos & Warren, 2007). Bird song is of particular interest to evolutionary biologists and behavioural ecologists because it is a fundamental biological signal for defending resources, recognising conspecifics and choosing a mate (Catchpole & Slater, 1995; Podos & Warren, 2007). Furthermore, geographical isolation facilitates the development of behavioural barriers to gene flow between isolated populations of the same species (Darwin, 1871 ; West-Eberhard, 1983; Foster, 1999). Therefore, studies of divergence of behavioural signals, along with ecological factors and genetics, have been critical to understanding how barriers to gene flow develop (Grant & Grant, 1997; Slabbekoorn & Smith, 2002; Edwards et al., 2005; Phillimore et al., 2006; Price, 2008).

Several explanatory hypotheses have been proposed to explain geographical variation in bird song through the interaction of song learning mechanisms and isolation (Podos & Warren, 2007). The causal factors supporting these hypotheses tend to be either 1) short term or 2) long term. A critical short term factor is likely to be founder events, i.e. when dispersing individuals from an established population start a new population at a new location (Baker & Jenkins, 1987; Baker et al., 2003; Baker et al., 2006). The effect of this founder event will be relative to the size of the established population, the song diversity existing within it and the number of dispersers. If a small number of founders disperse from a very large established population that contains a great deal of song diversity there might be a bottleneck effect, i.e. the dispersing birds will only carry a small sample of the song diversity that is present in the established population to the new location. Conversely, if the established population is small or contains little song diversity, or if a large number of birds disperse, the effect of this bottleneck will be less apparent because the founders will carry much of the song diversity with them to the new location. This is analogous to the loss of genetic variation following founder events (Briskie & Mackintosh, 2004). In both cases the diversity of the founding population (the "neck" of the "bottle") relative to the established population (the "bottle") is

critical in determining the song or genetic diversity of the new population, although the sex ratio and age composition of the founding population might also be important factors.

Long term effects that potentially generate geographical variation in bird song are more varied. Many bird species learn their songs from conspecifics (Catchpole & Slater, 1995; Podos & Warren, 2007; Price, 2008). However, learning errors can occur when songs are not accurately copied. This process of cultural mutation or drift provides a mechanism by which song diversity might change over time (Jenkins, 1977; Podos & Warren, 2007). This change might be neutral in large established populations but the effect is likely to be greater in small isolated populations. Genetic drift might also lead to song divergence in isolated populations (Podos & Warren, 2007). For example, syrinx mass is correlated with vocal frequencies and if genetic drift alters syrinx mass within a given population it is likely that frequency range will also shift (Podos et al., 2004). Selection pressures can also lead to geographical divergence in song. Cultural selection occurs when certain songs are favoured over others because they are more effective for communication (Podos & Warren, 2007). In several species cultural selection has been implicated as habitat dependent (Catchpole & Slater, 1995; Patten et al., 2004; Slabbekoorn, 2004) as low frequency slow songs are more effective in forested habitats while high frequency fast songs are favoured in open habitats (Catchpole & Slater, 1995). In Galapagos finches natural selection acts on bill morphology due to food availability and interspecific competition (Grant & Grant, 2008). This in turn constrains beak gape and vocal tract configurations during vocal production and as beak size increases trill rates and frequency bandwidths decrease (Podos, 2001; Podos & Nowicki, 2004; Huber & Podos, 2006). Sexual selection predicts that elaborate or complex songs, those that challenge developmental or performance capabilities and songs that enhance male-male communication will be favoured in some populations, particularly by females (Podos et al., 2004; Price, 2008). These sexually selected traits can vary widely between populations and might also result in geographic divergence in song (Podos & Warren, 2007).

An intimate knowledge of song development and population history is clearly invaluable in teasing apart the various mechanisms that can influence geographical variation in bird song. While the mechanisms of song development have been described for many species (Catchpole & Slater, 1995) population history is often difficult to discern. In continental populations varying levels of dispersal and immigration between populations, often over vast geographical distances, complicate resolution of population history. Isolated island

populations offer valuable opportunities for research (Baker & Jenkins, 1987; Baker et al., 2006) but founder events are still rarely detected (Clegg et al., 2002; Baker et al., 2003; Brunton et al., 2008). In contrast, translocated populations of birds offer unique opportunities for studying evolutionary processes (Parker, 2008), such as the development of geographical in song, because detailed records are kept of the size, source and age of founding populations (Lovegrove, 1996; Hooson & Jamieson, 2003).

My study species, the North Island saddleback or tīeke Philesturnus rufusater, provides an ideal model system for studying the evolution of geographical variation in song. The NI saddleback is an ancient passerine in the family Callaeatidae (Holdaway et al., 2001). Anthropogenic factors reduced the NI saddleback to a single population of approximately 500 birds on Hen Island, New Zealand, by 1910. Although common on that island, the single population was vulnerable to extinction so a series of successful conservation translocations were initiated in 1964 (Lovegrove, 1996; Parker, 2008). Each translocation induced bottleneck was severe with an average of 43 birds (range = 20-146, N = 15) translocated from donor populations estimated at 500-1000 birds at the time of translocation. These bottlenecks were further accentuated by variable post-release survival of translocated birds (mean = 56%, range 41-79%, N = 5) (Parker & Laurence, 2008). There are currently 15 populations of NI saddleback, including the natural population on Hen Island, a single population on Coppermine Island established through natural dispersal from nearby (c. 150 m) Whatupuke Island (Newman, 1980) and 13 populations established through translocation (see Figure 1, Chapter 1). NI saddleback are weak fliers incapable of crossing water gaps > 250 m (Newman, 1980) and with the exception of Coppermine and Whatupuke Islands there is no natural dispersal between any of the populations. Furthermore, DNA analysis indicates that even the population on Coppermine was founded by a small number of founders (Lambert et al., 2005), suggesting that dispersal between the two islands is a rare event.

NI saddleback are a particularly vocal species with three main categories of calls; loud chatter songs that are given by both sexes, sexually dimorphic quiet calls and male rhythmical song (MRS) (Jenkins, 1977; Higgins et al., 2006). The evolution of MRS is the focus of the work presented in this thesis as it is used for territorial advertisement, defence and mate attraction (Jenkins, 1977). Critically, MRS is a culturally transmitted signal that young male NI saddlebacks learn from their contiguous neighbours when they acquire a territory (Jenkins, 1977). However, this process of learning MRS from neighbouring birds is not always

accurate and changes in MRS can occur through learning errors, a process Jenkins (1977) referred to as cultural mutation.

Jenkins (1977) pioneering work identifying both the mode by which MRS is learnt and the evolution of new songs through cultural mutations was largely confined to a single population of NI saddlebacks on Cuvier Island. However, the processes Jenkins (1977) identified might also influence the evolution of NI saddleback song at a metapopulation level. In this thesis I exploit the detailed knowledge of NI saddleback population translocation history (essentially "forced" dispersal events; see Figure 1, Chapter 1) along with information on song learning mechanisms to address questions around the evolution of geographical variation in bird song. In particular I address how geographical variation in song might develop in NI saddleback and how this variation might manifest itself from both evolutionary and conservation biology perspectives. Furthermore, I predict that both the short term founder effects following initial translocation and subsequent population level cultural mutations provide a means by which geographical variation can develop between translocated island populations of NI saddleback.

Thesis outline

The primary aim of the thesis I present here is to use translocated populations of North Island (NI) saddleback or tīeke to investigate questions around the evolution of geographic variation in bird song. However, in describing the suitability of translocated systems for scientific research I also place translocation in a broader societal context. Therefore, while the cohesive theme of the thesis is based on translocated populations of NI saddlebacks each chapter is written as an independent scientific paper. This approach leads to inevitable repetition but this has been minimised where possible through reference to other chapters. The great majority of the work presented here is my own but four of the six chapters include input from others that warrants co-authorship. Their respective contributions are acknowledged below. The NI saddleback has generally been considered a subspecies (*Philesturnus carunculatus rufusater*) of the New Zealand saddleback but following work by Holdaway et al (2001) is increasingly cited as a full species (*P. rufusater*). I follow Holdaway et al (2001) throughout the thesis in referring to the NI saddleback as a full species, and add additional support to this conclusion in Chapter 5. The thesis structure is as follows:

<u>Chapter One:</u> This chapter uses translocated populations of NI saddlebacks as a case study to describe the multiple benefits that can accrue from translocations. The origins and motivation for modern translocations have their basis in applied conservation management and the process of creating new populations has obvious conservation outcomes. However, translocated systems also offer unique and invaluable research opportunities, particularly for evolutionary questions, because detailed records of the size, source and founding composition of translocated populations are usually available. The translocated populations of NI saddleback described in this chapter form the basis of subsequent investigation in this thesis. Finally, translocations provide a unique advocacy opportunity in that the general public can be involved in planning, implementation and post release monitoring. This aspect of the chapter might seem out of place in what is ostensibly a scientific thesis. However, it is not enough, for me at least, to only investigate purely scientific questions. As a field biologist I have a responsibility to contribute wherever possible to the conservation of biodiversity. Translocations achieve this by facilitating public participation in applied conservation and providing an outlet for communicating the scientific investigations that often accompany them. This chapter has been published as:

Parker, K.A. 2008. Translocations: Providing Outcomes for Wildlife, Resource Managers, Scientists and the Human Community. Restoration Ecology 16: 204-209.

<u>Chapter Two:</u> This key chapter demonstrates that significant geographic variation in song can rapidly develop through cultural evolution following conservation translocations. It is written in the concise form of a general science journal (e.g. Nature, Science, Current Biology, PNAs) and includes the following co-author contributions; I designed the sampling regime, conducted the field work, analysed the data and wrote the chapter with contributions from Marti J. Anderson, Peter F. Jenkins and Dianne H. Brunton. Marti J. Anderson (Massey University) provided significant guidance and input to the statistical analyses. Peter F. Jenkins collected the historical Cuvier data included in the analyses. Dianne H. Brunton conceptualised the project following the initial work of Peter F. Jenkins and also participated in field work.

<u>Chapter Three:</u> This chapter describes a translocation of NI saddleback from Tiritiri Matangi Island to Motuihe Island. This translocation has provided the outputs described in Chapter 1; it has created a new population of NI saddleback, there was significant community participation in all aspects of the translocation and it has subsequently been used for the

playback experiments that are the subject of Chapter 4. I led the translocation, the post-release monitoring and wrote the chapter. My co-author, John Laurence, leads the restoration of Motuihe Island through the Motuihe Restoration Trust which planned, funded and facilitated the translocation. This chapter has been published as:

Parker, K.A. and J. Laurence, 2008. Translocation of North Island saddleback *Philesturnus rufusater* from Tiritiri Matangi Island to Motuihe Island, New Zealand. Conservation Evidence 5: 47-50.

Chapter Four: This chapter uses a playback experiment on the Motuihe population described in Chapter 3 to test the impacts of the divergence of song amongst translocated populations described in Chapter 2. I designed the experiments, carried them out, analysed the data and wrote the paper with input from my co-authors Mark E. Hauber (City University of New York) and Dianne H. Brunton. This chapter has been published as:

Parker, K.A., M.E. Hauber, D.H. Brunton 2010. Contemporary cultural evolution of a conspecific recognition signal following serial translocations. Evolution 64: 2431-2441.

Chapter Five: This chapter assesses the role of vocalisations, morphology and mtDNA in defining the relationship between the NI saddleback and the closely related South Island (SI) saddleback (*Philesturnus carunculatus*). I collected all of the NI saddleback data used in the chapter, conducted all of the vocalisation and morphological analyses and wrote the chapter. Karen Ludwig (University of Otago) recorded the SI saddleback vocalisations. Ian J. Jamieson (University of Otago) and Tania King (University of Otago) collected the SI saddleback blood samples and conducted and interpreted the mtDNA analyses. These co-authors, along with Dianne H. Brunton, provided essential feedback whilst writing the chapter.

Chapter Six: This chapter draws general conclusions from the data presented in this thesis and speculates on the future role of song in isolated populations of translocated NI saddleback.

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Acknowledgements

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1 Translocations: providing outcomes for wildlife, resource managers, scientists and the human community

Conservation is getting nowhere because it is incompatible with our Abrahamic concept of land. We abuse land because we regard it as a commodity belonging to us. When we see land as a community to which we belong, we may begin to use it with love and respect.

-Aldo Leopold 1949 "A Sand County Almanac"

1.1 Abstract

The World Conservation Union (1987) defines a translocation as a release of animals with the intention of establishing, re-establishing or augmenting an existing population. Despite frequent use as a tool for the management of threatened and endangered wildlife, the full benefits of translocations often go unrealized. Here I demonstrate how translocations can achieve outputs for conservation management, conservation science and the wider human community, using North Island Saddleback or Tīeke (*Philesturnus rufusater*) as an illustrative example. From a conservation management perspective North Island Saddleback have been salvaged from a relic population of <500 birds on 484 ha Hen Island to a metapopulation of approximately 6,000 birds on 13 offshore islands and at two mainland New Zealand sites. These translocations have reduced the risk of global extinction for this species and helped restore the ecosystems involved. All of these translocations have occurred in the last 42 years from known source populations and with known numbers of birds released. The resulting replicated serial population bottlenecks provide numerous scientific opportunities for conservation and biological research. While the first Saddleback translocations were to reserves closed to the public, subsequent translocations have been to open reserves providing the wider human community with an opportunity to see, and be actively involved, in the management of a threatened endemic species. This has raised the profile of both North Island Saddleback and other species, and has provided wider community conservation benefits. These three outputs illustrate the value of translocations for resource management, conservation science and for increasing community interest, participation and investment in biological conservation.

1.2 Introduction

The World Conservation Union (1987) defines a translocation as a release of animals with the intention of establishing, re-establishing or augmenting an existing population. While the underlying premise of a conservation translocation is deceptively simple - animals are reintroduced to historic ranges or introduced to safe conservation areas – the potential benefits beyond establishing a new population are rarely explicitly addressed. And yet broad support and interest may be critical to the success of any translocation project (Brietenmoser 1998). Sarrazin and Barbault (1996) emphasize the research opportunities provided by translocations and Seddon et al. (2007) suggest that maximum progress will be made in the emerging field of reintroduction biology through the collaborative efforts of interdisciplinary teams of resource managers and scientists. The World Conservation Union guidelines for reintroductions (1995) call for local community consultation when planning a translocation, but there are few published accounts of how this might occur (Craig 1994; Galbraith & Hayson 1995). Further more, I suggest that meaningful community participation should be viewed as one of the primary outputs of a translocation, along with management and scientific objectives, rather than simply part of the planning process.

Resource managers are the traditional instigators of translocations but reintroduction biologists plays an increasingly vital role in research, planning and implementation (Seddon et al. 2007). However, both resource managers and scientists rely, to varying degrees, on public support for their continued funding and for approval of many translocations. Despite this, scientists are widely criticized for their inability to effectively communicate their research to the general public and more alarmingly, are often viewed with suspicion (Miller 2005; Meine et al. 2006; Robinson 2006). Resource managers also often suffer from poor public relations in that the news they deliver is often negative (humans are destroying our resources) and authoritative (therefore you cannot go there, touch this, harvest that) (Holling & Meffe 1996). Scientists and managers naturally tend to focus on the most interesting and innately satisfying part of the translocation process, that of establishing new populations and investigating the associated questions. But translocations provide opportunities that go beyond immediate management goals and science, opportunities that if ignored, could undermine the support we require from the wider community, and miss the full breadth of our responsibilities as conservation biologists. People increasingly live urban lives of biological poverty (Turner et al. 2004) prompting the argument that advocacy for the natural world may

be the most important role of conservation biology (Brussard & Tull 2007). Therefore, I propose that translocation projects are better viewed as multidisciplinary endeavors that deepen understanding, and forge valuable relationships amongst resource managers, reintroduction biologists and the wider community. I will demonstrate this using *Philesturnus rufusate*r (the North Island (NI) Saddleback or Tīeke), a threatened endemic New Zealand wattlebird whose conservation and translocation history has provided clear tangible outcomes for these three groups.

1.3 North Island Saddleback, conservation management, science and community participation

1.3.1 Conservation management

The New Zealand wattlebirds are ancient passerines belonging to the family Callaeatidae (Heather & Robertson 1996). They are represented by the extant NI Saddleback, South Island (SI) Saddleback (*Philesturnus carunculatus*) and North Island Kokako (*Callaeas wilsoni*) and the extinct South Island Kokako (*Callaeas cinerea*) and Huia (*Heteralocha acutirostris*) (Holdaway et al. 2001). They are all forest dwellers, poorly flighted, and have been reduced to remnant populations or extinction through anthropogenic factors (Heather & Robertson 1996). By 1910 the NI Saddleback was reduced to a single population of about 500 birds on 484 ha Hen Island. While NI saddlebacks were considered common on the island, it was apparent that a single population was extremely vulnerable to extinction (Merton 1973). Subsequently, there were three attempts to translocate NI Saddleback between 1925 and 1950 (Merton 1973; Lovegrove 1996a). These translocations all failed due to difficulties in capturing sufficient numbers of birds, unsuccessful captive maintenance and/or a limited understanding of the role of predators at release sites (Lovegrove 1996a). However, a further attempt in 1964 using mist nets, pre-recorded calls, speaker play back systems, improved captive maintenance and better understanding of release site requirements, led to the successful capture and translocation of 23 birds to Whatupuke Island (Merton 1973), resulting in a long-term established population. These techniques have been further refined and used in 30 subsequent translocations to 15 islands and three protected (predator proof fenced with intensive trapping and poisoning regimes) mainland New Zealand sites. There are currently 13 established island populations and two at protected mainland locations

(see Lovegrove 1996a; Hooson & Jamieson 2003 and http://www.massey.ac.nz/~darmstro/nz_projects.htm for reviews of Saddleback translocations). The extinction risk for NI Saddleback has been drastically reduced by moving from a small island population to a metapopulation of approximately 6000 birds (Hooson & Jamieson 2003). In addition to this highly desirable management goal the techniques tested and refined in NI Saddleback populations have been utilized for the recovery of many other New Zealand bird species (Lovegrove & Veitch 1994) particularly the SI Saddleback (Merton 1973).

Natural population	First level translocation	Second level translocation	Third level translocation	Fourth level translocation
Hen	**Whatupuke**	**Lady Alice**	**Little Barrier**	
Remnant population estimated at 500 birds	*1964 (23)*	*1971 (21)*	*1986 (42)*	
		Coppermine		
		Natural colonisation		
	Red Mercury			
	1966 (26)			
	Cuvier	**Stanley**	**Kapiti**	
	1968 (29)	*1977 (24)*	*1987 (43), 1988 (39), 1989 (40)*	
		Tiritiri Matangi	**Mokoia**	**Bushy Park**
		1984 (24)	*1992 (36)*	*(mainland) 2006 (36)*
		Little Barrier	**Karori**	
		1984 (50), 1987 (47), 1988 (49)	*(mainland) 2002 (40)*	
		Moutuhora	**Motuihe**	
		1999 (40)	*2005 (20)*	

Figure 1. The translocation history of the North Island saddleback showing the years of translocations and the sizes and sources of the founding population. The population on Little Barrier was founded from three different islands (Whatupuke, Lady Alice and Cuvier) and that on Coppermine is the only example of a natural colonization event. The Kapiti Island population was primarily founded by birds from Stanley Island. However, one bird from Hen, one from Lady Alice or Whatupuke and four birds from Cuvier survived from previous failed translocations (Lovegrove 1996a).

1.3.2 Conservation science

The scientific opportunity presented by NI Saddleback translocations was first realized by Jenkins (1977) who identified the cultural transmission of song patterns and dialects in a translocated population. Subsequent research has exploited individual translocated populations as well as examining effects across the NI Saddleback metapopulation. The

translation history of NI Saddleback is well documented (Figure 1), detailing founder population size, source population and date of establishment and reveals the opportunity for research into some of the most pressing questions in conservation biology, particularly the effect of serial population bottlenecks (Lambert et al. 2005). This provides tremendous opportunity both now and into the future for applied and pure research (Table 1). That is, while there is increasing concern and desire to manage the genetic composition of bottlenecked populations, leaving some populations relatively unmanaged creates research opportunities. Those populations resulting from serial bottleneck events (e.g. Hen – Cuvier – Tiritiri Matangi – Mokoia – Bushy Park) provide opportunities for controlled studies, where the long-term (>100 years) effects may be informative to the recovery of many species. The replicated bottleneck events also provide opportunities for long term controlled studies into concepts such as "genetic rescue" (Jamieson et al. 2007; Tompkins 2007). In addition to the obvious genetic questions (Table 1), NI Saddleback translocations have also facilitated research on fundamental ecological theory such as density dependent population growth (Armstrong et al. 2005) as well as applied research on population modeling (Armstrong & Davidson 2006) and release strategies (Armstrong & Craig 1995; Lovegrove 1996a) (Table 1). In addition to the published works (Table 1), there is current research at four institutions on the effects of translocation on song development, stress and sperm morphology.

Table 1. Scientific literature based on translocations of North Island saddleback. Only papers directly related to translocations have been included.

Research Area	Authors
Population models for reintroductions	Armstrong and Davidson 2006
Founder effects	Taylor et al. 2005
Serial population bottlenecks and genetic variation	Lambert et al. 2005
Population dynamics and density dependent effects	Armstrong et al. 2002; Armstrong et al. 2005; Brunton and Stamp 2007; Cassey et al. 2007
Nest box use in reintroduced populations	Stamp and Brunton 2002
The impacts of toxin use for restoration	Spurr 1993; Empson and Miskelly 1999; Davidson and Armstrong 2002
Release strategies for translocations and conservation status	Armstrong and Craig 1995; Lovegrove 1996a; Hooson and Jamieson 2003
Impacts of rat predation	Lovegrove 1996
Song development	Jenkins 1977

1.3.3 The wider human community

Early translocations of NI Saddleback were initiated and controlled by the New Zealand Wildlife Service but volunteers often played important roles in translocation teams (T.G. Lovegrove 2007, Auckland Regional Council, New Zealand, personal communication). Although these volunteer experiences led many participants to careers in conservation management and science the early translocations were to island reserves that were closed to the public, so only a privileged few were able to experience NI Saddleback in the wild. However, in 1984 birds were translocated to Tiritiri Matangi Island (Lovegrove 1996a; Hooson & Jamieson 2003), a scientific reserve that is open to the public. Although the translocation was carried out by the New Zealand Wildlife Service it was facilitated by the restoration efforts of volunteer planters and laborers (Rimmer 2004) thereby expanding the role of volunteer to one of habitat restoration and protection. The translocation has been an outstanding success and birds have successfully colonized most vegetated areas of the island (Craig 1994). The NI Saddleback release was not a public event but subsequent releases of other species to Tiritiri Matangi have involved the interested public in capture, release and subsequent monitoring of the translocated animals (Rimmer 2004). Crowds of up to 600 people have attended each release and releases have received extensive media coverage (Rimmer 2004). The NI Saddleback has gone on to become the emblem of the Tiritiri Matangi Supporters Incorporated, a community group established with the intent purpose of supporting and actively participating in the restoration and maintenance of Tiritiri Matangi Island.

The public involvement in the restoration of Tiritiri Matangi has led to many other community based restoration projects. Of these, the Karori Wildlife Sanctuary (www.sanctuary.org.nz), the Motuihe Restoration Trust (www.motuihe.org.nz) and Bushy Park Sanctuary (www.bushypark.co.nz) have restored habitat and initiated, funded, implemented and subsequently monitored NI Saddleback translocations (Figure 1). These three translocations are unique among NI Saddleback translocations in that they are fully community based initiatives. Indigenous peoples also often have a strong desire to reassert traditional management of natural resources (Taiepa et al. 1997), and in New Zealand the gifting of wildlife as a *taonga* (treasure) from one Māori *Iwi* (tribe) to another is a significant event. In addition, Māori approval is a legislative requirement for a translocation to proceed in New Zealand (Department of Conservation 2004). Involvement in saddleback

translocations has often been restricted to blessing the released birds and acknowledgement of movement from one *Iwi*'s rohe (area) to another, but there is extensive involvement in planning and monitoring of other species (Moller et al. 2004). Reconnecting people with the natural world is widely acknowledged as one of the greatest challenges facing conservation biology (Turner et al. 2004; Leopold 2004; Balmford & Cowling 2006; Bearzi 2007; Paquette 2007). People develop understanding and empathy from experience (Leopold 2004; Brewer 2006) and these translocations have offered a tangible process for involving, educating and encouraging the wider community. They provide a continuum of experiential opportunity for people of varying levels of interest, from restoring a site for translocation, through to capture, release, and contact with the translocated population. During involvement in 15 avian translocations and the associated restoration projects I have witnessed first hand the impact they have on participants and visitors, many of whom become passionate advocates for restoration and translocation. In New Zealand there has been a proliferation of community initiated restoration projects over the last two decades. The community groups are largely voluntary (Galbraith & Hayson 1995) but they understand the problems, and are motivated to act. Their short-term goals may be to reverse and mitigate local degradation, but for many the long-term motivation is translocation of extirpated species.

Those directly involved in community restoration projects have an obvious sympathy for conservation. But what of those whose involvement is limited to the taxes they pay? Turner et al. (2004) state that the number and variety of people aware of and involved in the integration of biodiversity must grow. It is here that the media can play an important role, both in educating the general public, and as a catalyst for some to seek direct conservation experience and involvement. The media recognize the news worthiness of translocations, the sound bites and images a welcome antidote to the doom and gloom that is rampant in modern news reports. Despite an increasingly urbanized populace out of touch with the natural world they inhabit, many people respond to animals (Mankin et al. 1999; Muth & Jamieson 2000). Therefore, by facilitating media coverage, resource managers, scientists and community groups can raise both the profile of their particular projects, and of conservation as a whole.

1.4 Realizing the synthesis

While the benefits of viewing translocations as providing multiple outputs are clear, actually implementing the synthesis is challenging. A resource manager's reluctance to relinquish full control of the translocation process may be justified in the face of ill prepared or premature

proposals from scientists, community groups, and even fellow managers. Clear communication of minimum requirements for the successful establishment of new populations is often still lacking and urgently required. Decisions need to be explained and justified, with great care taken to distinguish between scientific or technical issues and value judgments (Lackey 2007). This information will primarily be vertically transmitted from resource managers and scientists to community groups and other scientists. However, expectations need to be communicated in both directions, particularly those of communities adjacent to release sites. Translocations can be seriously compromised by local community opposition, as demonstrated by many attempts to reintroduce large mammals (Breitenmoser 1998), and historically poor communication by resource managers can complicate contemporary translocation efforts (C.M. Miskelly, 2007, Department of Conservation, New Zealand, personal communication).

In contrast to the vertical transmission of biological requirements for a successful translocation, horizontal transmission of funds, labor, motivation and ongoing commitment can occur across all three groups. Community groups in particular can be a significant source of funding and labor for protecting large tracts of habitat. The New Zealand based Maungatautari Trust has raised over 14 million New Zealand dollars for the predator proof fencing and subsequent protection of a 3,363 ha forested site, which will facilitate the translocation of a diverse range of plant and animal species (www.maungatrust.org). Such community actions have the potential to accelerate the growth of protected areas for restoration and translocation, free of the resource restrictions implicit in government based efforts. While promoting the opportunities available for translocation, I do not advocate a complete devolution of traditional structures of resource management. The skills and knowledge required for a successful translocation that are traditionally held by professional resource managers and scientists will always be the main force behind translocations, particularly of endangered species. However, regardless of who undertakes a translocation these skills and knowledge are essential for the welfare of the translocated animals and the success of a project.

Translocation is a particularly relevant tool within New Zealand conservation, but similar benefits have been realized in other countries by considering local communities as integral to conservation success. Horwich and Lyon (2007) describe 23 projects in 9 countries, both developed and developing, where active community participation has been a key component

of success. Two of these projects have involved translocations and 15 community groups have formed as a result of others. Large scale community conservation efforts have been criticized, particularly in developing countries, for not delivering to local communities (Musumali et al. 2007). However, Horwich and Lyon (2007) make a useful distinction between large scale integrated conservation and development projects (ICDPs), and small community based initiatives. They argue that small scale community conservation projects that encourage equality, independence and empowerment represent one of our best chances for conservation success and call for a refocus on low budget, community scale projects.

Innovation and constant self assessment are vital for effective conservation management and science. We must actively seek new opportunities for partnerships, participation and funding not only for translocations, but in all our conservation endeavours, thereby increasing understanding, support, and the effectiveness of our efforts. By loosening traditional views and management structures, and recognizing the diverse politics involved in protecting species, all parties will be prompted to take greater responsibility not only for translocated species, but for habitat restoration and biological conservation in general.

1.5 Implications for practice

- Translocations are best optimized by treating them as multi-disciplinary endeavors that deepen understanding and forge valuable relationships among resource managers, scientists and the general community.

- Increasing community participation and awareness should be viewed as a primary translocation output rather than just a component of the planning process.

- The general public is critical to the success of conservation and restoration, and yet are increasingly detached and isolated from the natural world. Translocations provide a unique opportunity to reconnect.

- Translocations and restoration can be better planned, funded, implemented and maintained by increasing their broad support base.

1.6 Acknowledgements

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2 Conservation translocations cause rapid cultural evolution of bird song

There is a geographical quality to listening

-Dianne Ackerman 1990 "A natural history of the senses"

2.1 Abstract

Geographical isolation plays a crucial role in speciation events[1]. Thus studies of divergence of behavioural signals in isolated populations, along with ecological factors and genetics, have been critical to understanding how barriers to gene flow develop[1-5]. Bird song is a vital conspecific recognition signal (CRS) and many studies have demonstrated significant geographical variation in song with several hypotheses posed to explain this variation[6,7]. However, a key problem in testing these hypotheses is an inability to measure the pace of song divergence. This is because the timing and source of founder events are rarely detected[8]. Conservation translocations provide exceptional opportunities for studying evolutionary questions because the age and source of founder populations are completely known[9]. Here we use the North Island (NI) saddleback *Philesturnus rufusater*, isolated on a single island in 1964 but subsequently increased by translocation to 13 island populations[9,10], to show that significant geographical variation in song can develop in less than 50 years. Furthermore, our data show a clear signal of serial population bottlenecks (up to 3 times) following translocation and support both bottleneck and cultural mutation hypotheses[11] in explaining this variation. Critically NI saddleback discriminate between songs from different islands[11] and this discrimination might lead to an eventual reduction in effective population size[12]. This illustrates the potential for human induced founder and isolating events, including conservation management, to be microevolutionary events and challenges us to consider the implications of conservation biology in an evolutionary context[13].

2.2 Results and Discussion

Geographical isolation facilitates the development of behavioural barriers to gene flow[3,5,14]. Geographical variation in bird song, an important conspecific recognition signal (CRS), has

been particularly well studied[4,5] and several hypotheses have been proposed to explain this variation through the interaction of song learning mechanisms and isolation[1,15]. Causal factors supporting these hypotheses fall into two broad categories[16]: 1)short term effects, e.g. founder events, and 2) changes that occur over a longer period, e.g. natural selection[15,16]. Therefore a critical question is how long does it take for geographical variation in bird song to develop? In continental populations[6,17] varying levels of dispersal and immigration between populations make resolution of this question difficult. Isolated island populations offer valuable opportunities for research[18,19] however they still require assumptions regarding the age, source and composition of founder populations. In contrast conservation translocations offer unique opportunities for studying evolutionary processes[9] because detailed records are kept of founder events. Here we use translocated populations of NI saddleback to study the tempo and processes by which CRS evolve. This provides a unique system because 1) NI saddleback communicate using male rhythmical song (MRS) a culturally transmitted CRS that is subject to change through learning errors[20], 2) they have been successfully translocated 14 times since 1964[10] and critically, 3) the size, source and age of the founding population on 11 of 13 islands (Figure 2a) is completely known[9,10].

Given our detailed knowledge of NI saddleback biology and population history we are interested in how quickly geographical variation in song can develop and propose two alternative hypotheses to explain this change. First, the bottleneck hypothesis predicts 1) any observed diversity between populations in MRS is a result of cultural bottlenecks when founded through translocation 2) a reduction in song diversity moving from the ancestral population through the translocated populations, particularly through serial translocations and 3) some MRS sharing between populations. Second, the cultural mutation hypothesis, predicts that 1) the accumulation of learning errors through cultural mutations leads to MRS divergence, and 2) little or no difference in song diversity among isolated populations with different translocation histories, depending on the rate of divergence.

A rapid (<50 years) effect of translocation is evident when song diversity is compared between islands. Non-metric multi-dimensional scaling (MDS) demonstrates clear decreases in the level of song sharing with increasing distance from the ancestral population (Figure 3a) when 2657 MRS from all 13 island populations of NI saddleback (Figure 2a) and historical recordings from Cuvier Island are compared. Effects of bottlenecks in translocation are significant either with (ANOSIM, $R = 0.30$, $P = 0.016$, 9999 permutations) or without
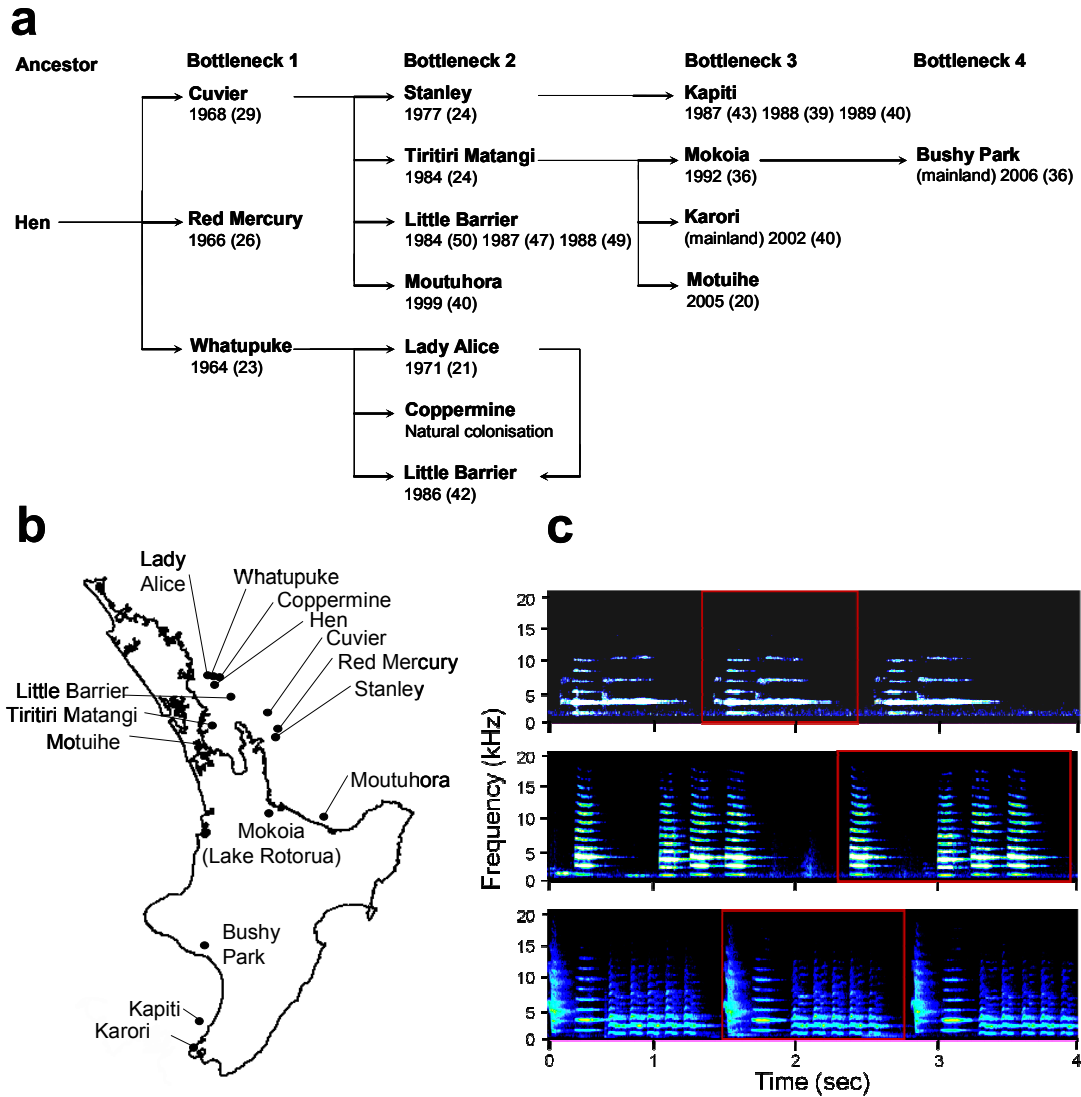
Figure 2. a) The translocation history of the NI saddleback in New Zealand showing the years of translocations and the sizes and sources of the founding population. The population on Little Barrier was founded from three different islands (Birds from Cuvier were released in the southwest corner of Little Barrier; Birds from Whatupuke and Lady Alice were released at P Flat) and that on Coppermine is the only example of a natural colonization event. The Kapiti population was primarily founded by birds from Stanley. However, one bird from Hen, one from Lady Alice or Whatupuke and four birds from Cuvier survived from previous failed translocations[18]. The two mainland populations (Karori and Boundary Stream) are not included in the analyses presented here. b) Geographic locations of NI saddleback populations. c) Spectrograms showing MRS (top to bottom) from Hen, Cuvier and Mokoia Islands. The red outlines show a single phrase as measured for spectral analysis.

(ANOSIM, $R = 0.31$, $P = 0.021$, 9999 permutations) historical MRS from Cuvier. In addition, multivariate dispersion (MVDISP), a measure of song diversity, decreased when moving from first order bottlenecks (relative dispersion = 1.28 with Cuvier historical, 1.57 without) to second (0.97, 0.97) and third order bottlenecks (0.62, 0.64), supporting previous work showing a reduction in song sharing relative to the point of origin[18,21].
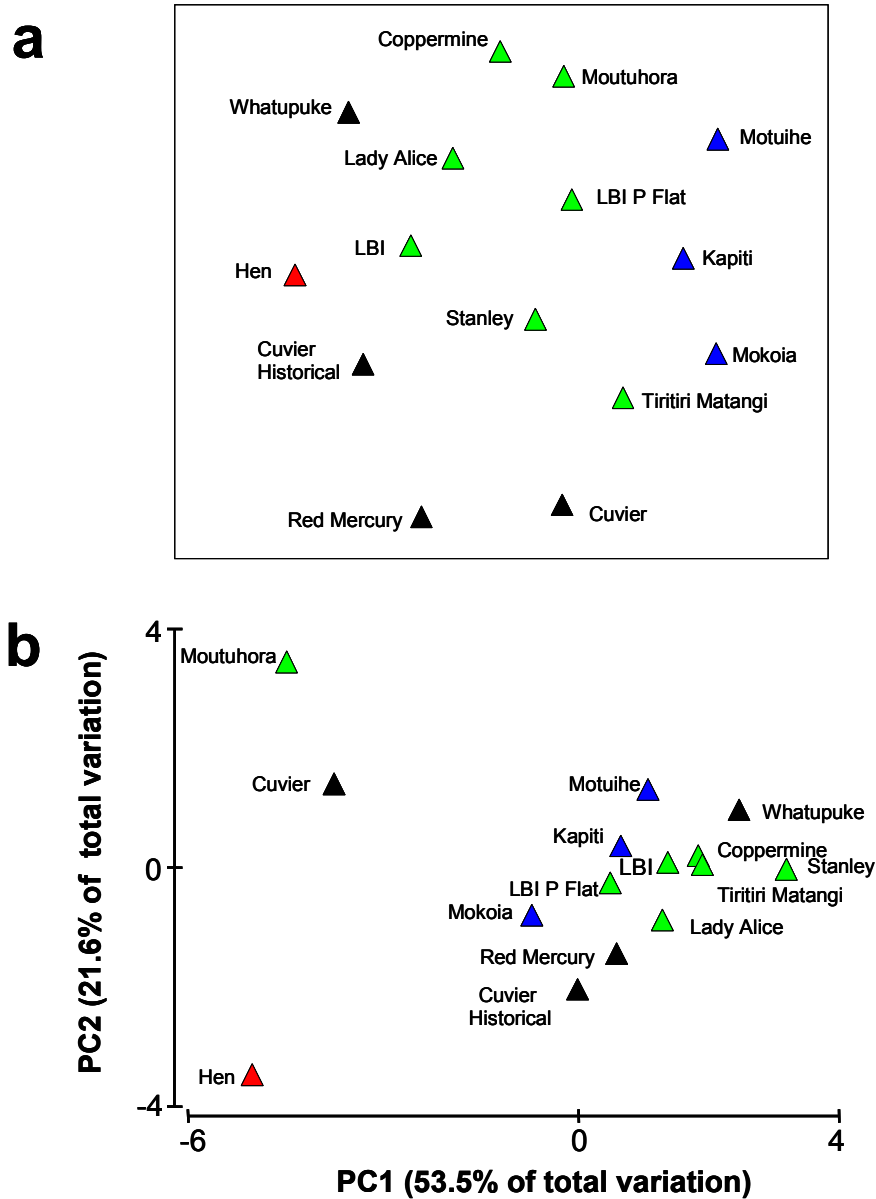
Figure 3. a) Non-metric MDS ordination showing patterns in MRS type similarities among islands in NI saddleback (2D stress = 0.22). These patterns are also shown in the lower stress 3D MDS ordination (3D stress = 0.13). b) PCA ordination of the average normalised spectral variables showing islands and bottleneck level. See **Table 2** for PC loadings (red triangle = ancestral population; black triangle = first-level translocations; green triangle = second-level translocations; blue triangle = third-level translocations).

Principal components analysis (PCA) of MRS spectral characteristics also demonstrates changes due to bottlenecks (Figure 3b; Table 2). Euclidean distances based on five spectral variables had maximum correlation (Table 3) with the MRS type similarities among islands (BIOENV, $|\rho| = 0.26$). Although these five variables explained just 40% of the variation in the MRS type similarity matrix (DISTLM), linear combinations of the spectral variables in a constrained ordination (dbRDA) preserved the essential pattern of decreasing similarity with increasing level of bottleneck (Figure 4a; Table 3), as seen in the unconstrained MDS (Figure 3a). There is a highly significant decrease in multivariate dispersion of spectral characteristics (PERMDISP, $F = 13.15$, $P = 0.0001$, 9999 permutations) when moving from the ancestral population through bottlenecks 1, 2 and 3 (Figure 4b). Thus, not only do songs change in character, they also decrease in spectral diversity with serial bottlenecks. There is also a clear bottleneck effect when spectral variables are examined individually (Figure 5a – 3e)

Cultural bottlenecks have clearly led to a rapid loss of song types between islands following serial translocations. However, MRS types unique to a single island comprise 77 % of the overall observed MRS diversity ($N = 202$). This supports the cultural mutation hypothesis. Consistent with prior research[18,22], we speculate that translocation disrupts the social system of NI saddleback, particularly the mode by which males learn MRS, leading to an increase in the cultural mutation rate and the subsequent emergence of new MRS through a withdrawal from learning process[1]. In established populations territorial vacancies are rare and rapidly filled by local recruits[23] who learn the MRS sung by their contiguous neighbours[20]. However, after initial translocation population density is extremely low, territorial space abundant and density-dependent juvenile survival is high[23]. Therefore young birds often establish isolated territories without contiguous neighbours and in the absence of adult MRS tutors. We speculate that these birds innovate MRS from the template offered by the innate chatter call[20] leading to the emergence of new MRS patterns within the population. However, the common chatter template provides a mechanism for MRS that are structurally and spectrally similar. This is consistent with our data showing reduced dispersion in spectral characteristics of bottlenecked populations (Figure 4b) and a reduction in song sharing (Figure 3a) with increasing bottleneck. The role of sexual selection in shaping MRS is unclear and while females may preferentially select certain MRS, this selection might also be relaxed in new populations if the cost of not finding a preferred mate is not mating at all[24].

Table 2. Principal component loadings for the analysis of spectral variables from island populations of NI saddleback.

Spectral variable	PC1	PC2
Log duration	0.358	-0.115
Log mean pitch	0.256	-0.347
Mean frequency modulation	0.278	0.183
Mean amplitude modulation	0.183	-0.098
Mean entropy	0.344	0.201
Log mean goodness of pitch	0.141	0.539
Log mean frequency	0.382	-0.027
Log variance in pitch	0.353	-0.228
Variance in frequency modulation	0.288	0.271
Log variance in entropy	0.267	0.028
Log variance in goodness of pitch	-0.047	0.602
Log variance in frequency	0.362	-0.040

The relationship between gene flow and geographic variation in song remains unclear. Some studies have found a correlation between song dialects and restricted gene flow[17,25] and others have not[26]. A genetic signal consistent with bottleneck history is evident in NI saddleback populations, but there is also a general pattern of low genetic diversity[27]. Correlations between habitat structure and spectral characteristics of songs have been demonstrated in several species[28]. We have not tested for this correlation, but the diversity of habitats utilised by NI saddleback are well represented both on the ancestral island (Hen) and within each bottleneck level (Figure 2a).

The cultural bottleneck and cultural mutation hypotheses are not mutually exclusive and our data, along with prior research[11,18,22], demonstrate support for both. Significantly, we know of few wild population studies with such detailed information on the pace and means by which changes in CRS might emerge and none where such a significant change in bioacoustical behaviour has been caused by intentional conservation management[29]. However, the critical point is that the evolutionary impact of geographical variation in CRS ultimately depends on patterns of signal perception and discrimination[7]. Many studies have demonstrated discriminatory behaviours in playbacks of familiar versus unfamiliar song[6,7,28] and this pattern is evident in Motuihe NI saddleback, which showed significant discriminatory behaviours when exposed to Motuihe MRS versus Hen or Cuvier MRS (Figure 2a)[11].
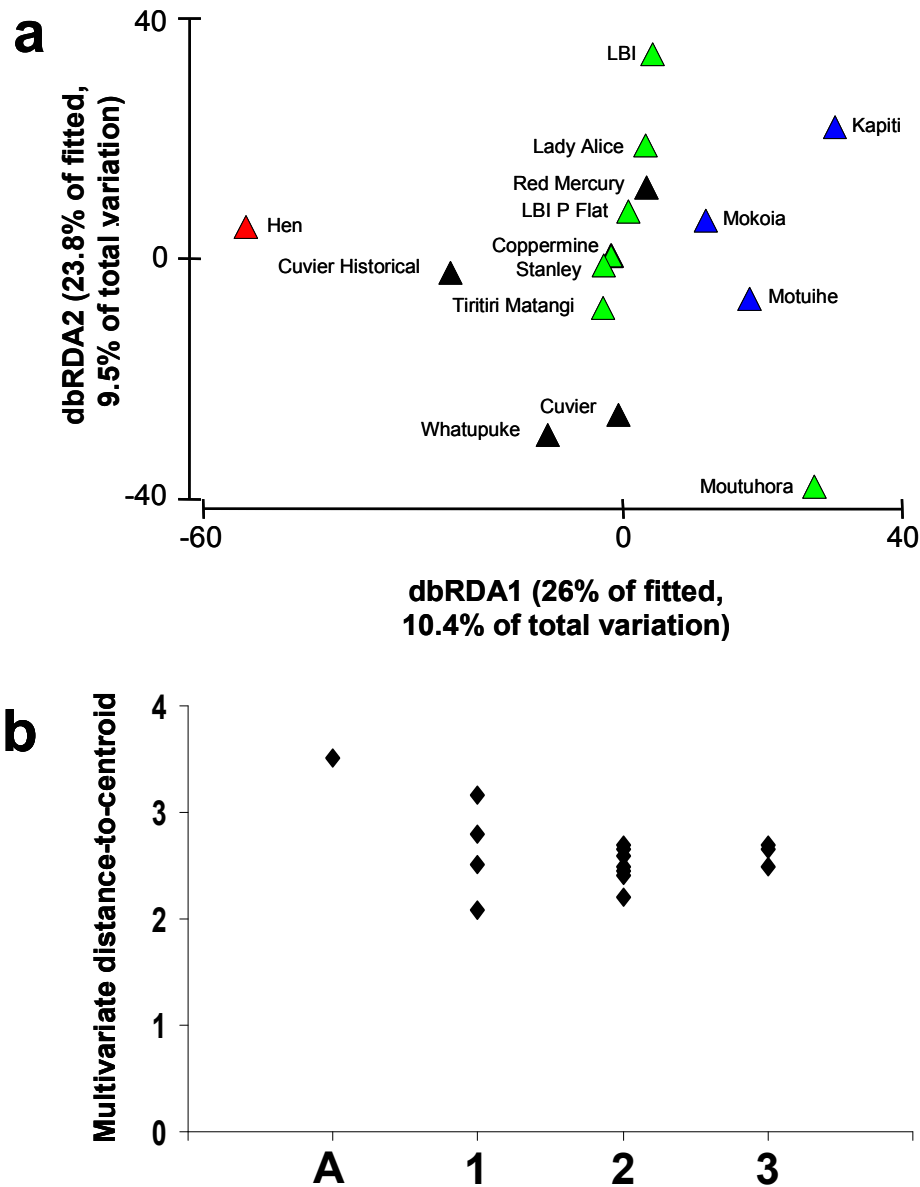
Figure 4. a) dbRDA ordination of MRS type similarities versus five spectral variables (log variance in goodness of pitch, log mean goodness of pitch, variance in frequency modulation, log variance in entropy and mean amplitude modulation. See **Table 3** for dbRDA correlations with spectral variables). (red triangle = ancestral population; black triangle = first-level translocations; green triangle = second-level translocations; blue triangle = third-level translocations).b) Multivariate distance-to-centroid of spectral variables within islands (dispersion) *versus* translocation level.

Table 3. Relationships between dbRDA coordinate axes and orthonormal spectral predictor variables (multiple partial correlations).

Spectral variable	dbRDA1	dbRDA2
Log mean goodness of pitch	0.895	-0.014
Log variance in entropy	0.240	0.726
Variance in frequency modulation	-0.115	-0.379
Log variance in goodness of pitch	0.348	-0.561
Mean amplitude modulation	-0.088	0.120

Slabbekoorn and Smith[5] proposed a model of acoustic divergence with song learning whereby both realised song diversity (the songs actually sung in a population) and potential song diversity (the songs individuals within a population are physiologically capable of singing) diverge over time. We suggest NI saddleback fit in the early stages of this model: there are clear shifts in the realised song diversity between island populations but all birds likely remain physiologically capable of producing MRS patterns present within other populations. NI saddleback might gradually proceed towards the latter stages of this model with song becoming a definitive population marker following divergence of potential acoustic variation between populations. This may be a relatively rapid process given that both founder populations (Figure 2a) and islands (135-3000 ha) are small[10] and dispersal between the populations is not possible.

Ultimately, the evolution of behaviour, along with genetic drift and ecological factors prompting natural selection, may lead to an allopatric model of speciation within translocated populations of NI saddleback[1,5]. An oft-stated goal of conservation biology is to preserve evolutionary potential but conservation practice can also direct evolutionary trajectories[13]. This is especially so where conservation mediated changes in behaviour might lead to an eventual reduction in the effective population size of managed species[12]. Speciation in birds proceeds with the evolution of behavioural barriers to gene flow[4]. The rapid divergence of CRS might be an early indicator of this process.
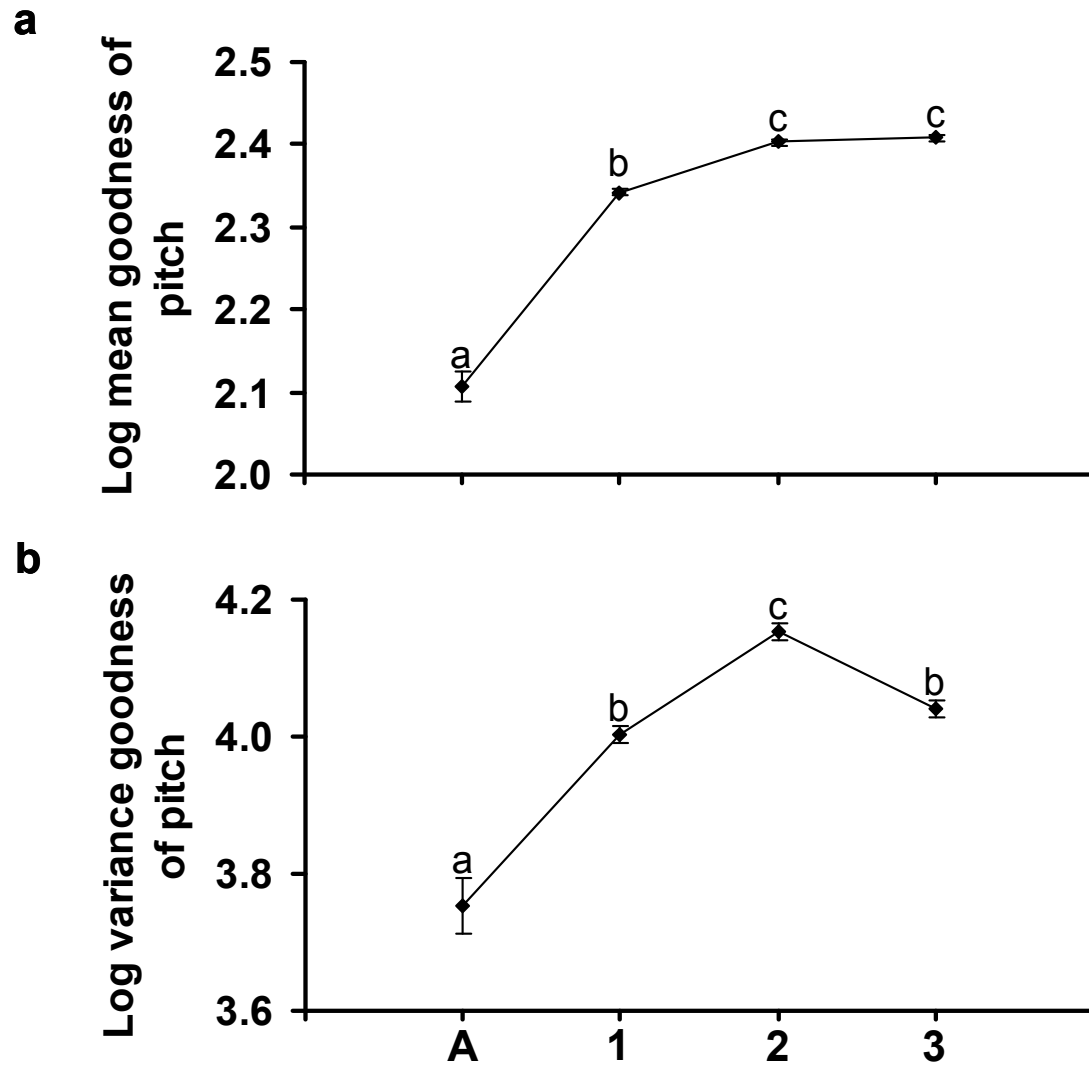
Figure 5. a-b) Mean ± 1SE of two of five individual spectral variables used for the dbRDA ordination. See Tchernichovski[30] for a full description of each spectral variable. A = ancestral population; 1 = first-level translocated populations; 2 = second-level translocations; 3 = third-level translocations. Differences in the lower-case letters (a-d) denote means which differ significantly from one another ($P < 0.05$) using Tukey's HSD comparisons following one-way ANOVA.
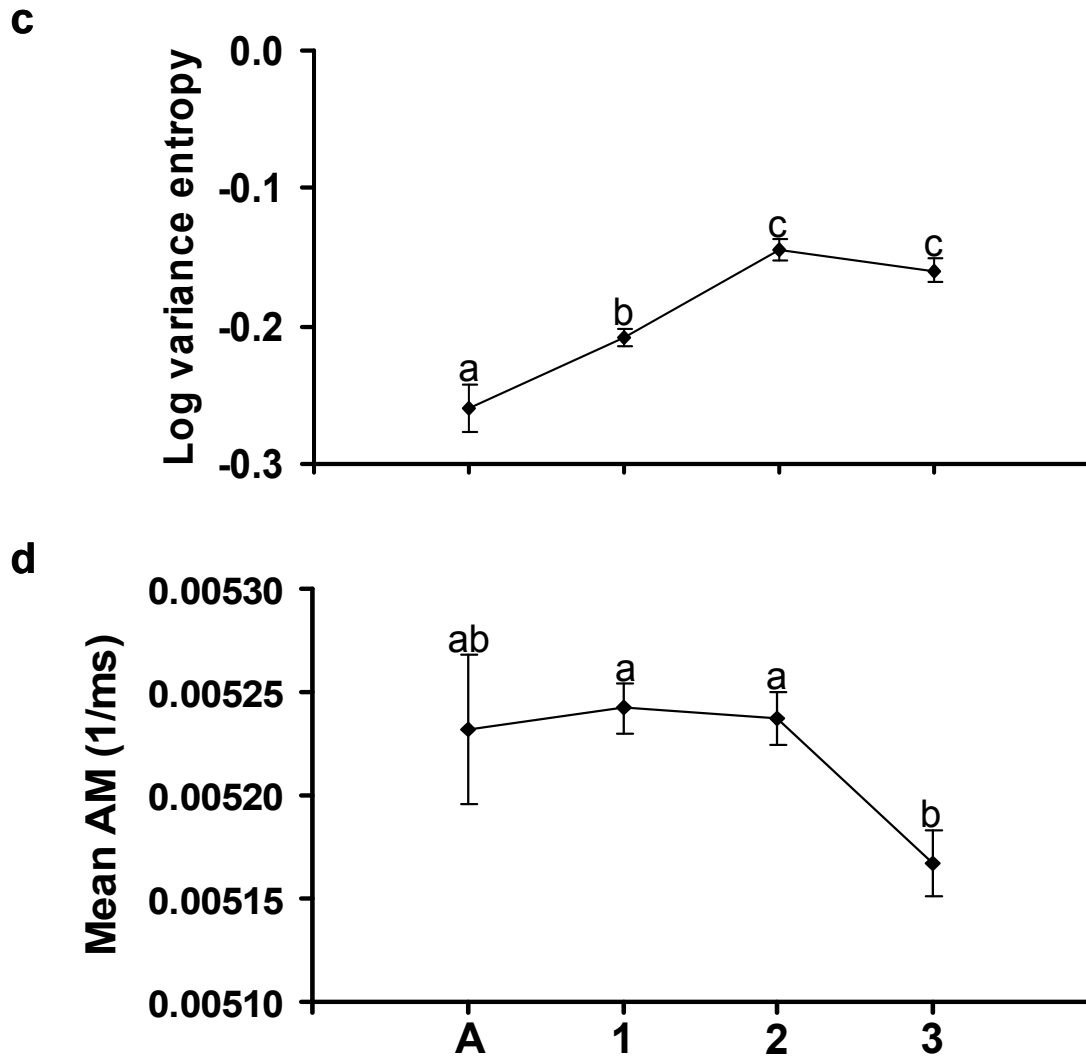
Figure 5. c-d) Mean ± 1SE of two of five individual spectral variables used for the dbRDA ordination. See Tchernichovski[30] for a full description of each spectral variable. A = ancestral population; 1 = first-level translocated populations; 2 = second-level translocations; 3 = third-level translocations. Differences in the lower-case letters (a-d) denote means which differ significantly from one another ($P < 0.05$) using Tukey's HSD comparisons following one-way ANOVA.
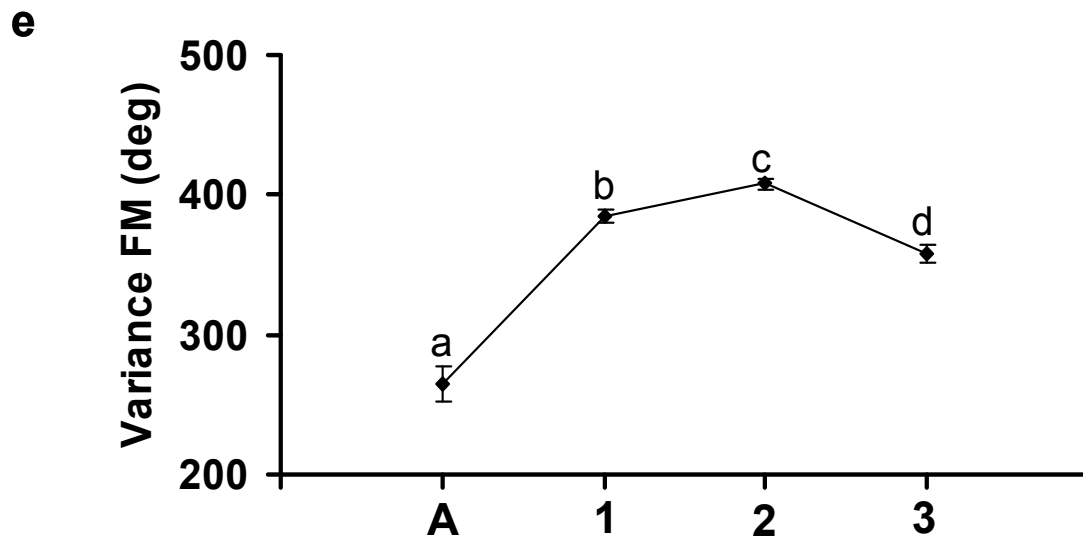
Figure 5. e) Mean ± 1SE of one of five individual spectral variables used for the dbRDA ordination. See Tchernichovski[30] for a full description of each spectral variable. A = ancestral population; 1 = first-level translocated populations; 2 = second-level translocations; 3 = third-level translocations. Differences in the lower-case letters (a-d) denote means which differ significantly from one another ($P < 0.05$) using Tukey's HSD comparisons following one-way ANOVA.

Table 4. Descriptive statistics (means ± 1SE) for spectral variables measured from MRS from the ancestral NI saddleback population, and the first, second and third level translocated populations. Differences in the lower-case letters (a-d) denote means which differ significantly from one another ($P < 0.05$) using Tukey's HSD comparisons. The Bonferroni adjusted experiment-wise significance level for conducting 12 simultaneous test is $0.05/12 = 0.00417$.

Spectral variable	Translocation level				Between site comparison	
	Ancestral ($N = 144$)	1st level ($N = 932$)	2nd level ($N = 925$)	3rd level ($N = 625$)	$F_{(3, 2622)}$	P value
Log duration	2.79^a ± 0.01	2.99^b ± 0.01	2.98^b ± 0.01	3.06^c ± 0.01	71.32	<0.0001
Log mean pitch	2.98^{ab} ± 0.01	2.98^a ± 0.00	2.94^c ± 0.00	2.97^b ± 0.00	30.18	<0.0001
Mean frequency modulation	11.29^a ± 0.61	17.36^b ± 0.25	17.42^b ± 0.23	15.24^c ± 0.28	39.91	<0.0001
Mean amplitude modulation	0.01^{ab} ± 0.00	0.01^a ± 0.00	0.01^a ± 0.00	0.01^b ± 0.00	5.45	< 0.001
Mean entropy	-5.07^a ± 0.05	-3.84^b ± 0.03	-3.73^c ± 0.02	-3.87^b ± 0.03	143.41	<0.0001
Log mean goodness of pitch	2.11^a ± 0.02	2.34^b ± 0.00	2.40^c ± 0.00	2.41^c ± 0.00	241.21	<0.0001
Log mean frequency	3.25^a ± 0.00	3.48^b ± 0.00	3.46^c ± 0.00	3.52^d ± 0.00	255.21	<0.0001
Log variance in pitch	5.39^a ± 0.01	5.67^b ± 0.01	5.54^c ± 0.02	5.65^b ± 0.02	29.11	<0.0001
Variance in frequency modulation	265.03^a ± 13.09	384.32^b ± 4.55	407.65^c ± 4.33	358.04^d ± 6.17	48.89	<0.0001
Log variance in entropy	-0.26^a ± 0.02	-0.21^b ± 0.01	-0.14^c ± 0.01	-0.16^c ± 0.01	23.83	<0.0001
Log variance in goodness of pitch	3.75^a ± 0.04	4.00^b ± 0.01	4.15^c ± 0.01	4.04^b ± 0.01	62.92	<0.0001
Log variance in frequency	4.29^a ± 0.11	5.14^b ± 0.02	5.08^b ± 0.02	5.33^c ± 0.02	103.18	<0.0001

2.3 Methods

2.3.1 North Island saddleback translocations.

19 successful saddleback translocations were conducted from 1966 to 2006 (Figure 2a and b)[9,10,31]. Most populations were established with a single translocation averaging 29 birds (range 20-40, $N = 12$) but Kapiti and Little Barrier received >100 birds in 3-4 translocations from several locations (Figure 2a and b). Where monitored post-release survival of birds to one year was variable (mean = 56%, range 41-79%, $N = 5$)[32].

2.3.2 North Island saddleback song.

NI saddlebacks give three distinct vocalisations; loud chatter calls, sexually dimorphic quiet calls and male rhythmical song (MRS)[20].

2.3.3 Song recording on islands.

Each of the 13 established island populations (Figure 2a and b) was visited for 5-15 days on 1-4 occasions between December 2004 and November 2008. MRS was sampled by 1-4 researchers with Sony Hi MD MZ-NH700 mini disc recorders, Sennheiser K6 recording modules and M66 microphones and Rycote Softy wind guards mounted on Rycote pistol grips.

During recording sessions on islands, the entire area was arbitrarily divided between researchers using tracks and natural features. All islands were sampled exhaustively with the exception of Little Barrier (3083 ha) which was sub-sampled in the southwest and northeast quarters of the island. These two areas were kept separate in analyses as they were established from different translocation events (Figure 2a). Each researcher would move through their assigned area searching for birds giving MRS' which were recorded for up to 30 minutes, along with location details and behavioural observations. Recordings were made throughout the day with greatest effort sunrise to 1130 h and 1430 h to sunset.

All discs were manually transcribed and each MRS given a unique file name before downloading and conversion to WAV. format using Sony SonicStage software (Sony Corporation 2001-2007) with 16 bit sampling precision and a 44 kHz sampling rate.

2.3.4 Historical Cuvier Island recordings.

Historical Cuvier Island recordings were collected from 1970 to 1972 using an Uher Report L tape recorder with an Uher 514 omni-directional microphone. They were digitized by the Macaulay Library of Natural Sounds, Cornell Lab of Ornithology, in 2005 with 16 bit sampling precision and a 44 kHz sampling rate. The digitization process was a straight duplicate and there are no apparent artefacts from this process or from the original analogue recordings (*e.g.* recording speed errors or abnormal harmonic content (*pers. comm.*, G. Budney and M.J. Anderson, Cornell Lab of Ornithology).

2.3.5 MRS categorisation.

NI saddleback MRS' are easily distinguished both visually and aurally (Figure 2c) and this remains one of the most sensitive methods of categorising bird song type[6]. All recordings (N = 2657) were subsequently inspected (FFT = 256, Hann window, 5.8 ms, 50% overlap) using Raven 1.3 (Cornell Lab of Ornithology, USA) to assign recordings to MRS type. Initial sorting was within islands as identical MRS' were often recorded sequentially from either an individual male or groups of males. A reference file was made from representative examples of these recordings by copying a spectrogram image from Raven into a MS Word document. Each individual MRS sound file was opened in Raven and visually compared to a hard copy of the Word reference file of all other MRS from the same island. We were necessarily conservative in our song matching but made allowances for individual variation present within islands, *i.e.* variation in the tempo or pitch of delivery or the number of repeated syllables within a phrase. If a match was located the matching sound file was opened in Raven and aurally and visually compared to the sound file being categorized. This reduced the MRS diversity within an island to a single MS Word reference file containing one spectrogram of each MRS present on the island (mean MRS per island = 20, range 7-40, N = 300). The process was then repeated by individually comparing each MRS to all other MRS recorded on all islands, thereby reducing the data set down to MRS that are either shared with >1 island (N = 47 shared MRS) or only found on 1 island (N = 155 MRS). Each of the MRS (N = 202 unique MRS) was then given a new file number and entered into a spreadsheet listing the islands on which it was present and the number of individuals that were recorded singing the MRS within each island (mean number of individuals recorded per island = 45, range = 12-115, N = 15 sites for a total of 681 individuals). No significant differences in song matching scores ($F_{2,36}$ = 0.84, P = 0.44) were found when 2 independent observers (1 an

experienced bird song researcher, the other with no prior experience analysing song) sorted a random sample of 50 MRS' to MRS type (there were 13 different MRS types within the random sample). Sampling effort was assessed by plotting the number of new MRS observed against the number of new individuals recorded. On all the islands the song accumulation curve approached an asymptote indicating that the majority of song types had been sampled.

2.3.6 Spectral measurements.

Spectral variables (duration and both the mean and the variance of pitch, entropy, frequency modulation, amplitude modulation, goodness of pitch and frequency) were measured from a single phrase (Figure 2c) within each MRS in Sound Analysis Pro[30] (Version 1.02) using the manual segmentation procedure with a 200 Hz high pass filter (data window = 9.27 ms, advance window = 1.36 ms, spectrum range = 11 180 Hz, Fast Fourier Transform window = 1024 samples, tapers = 2, bandwidth parameter = 1.5). The data were examined for normality and the following variables were transformed to $\ln(x)+0.01$ to remove skewness: duration, mean pitch, mean entropy, mean goodness of pitch, mean frequency, variance in pitch, variance in entropy, variance in goodness of pitch and variance in frequency. Extreme outliers (1.2%, N = 31 MRS) were removed prior to subsequent analysis.

2.3.7 Statistical analyses.

All multivariate analyses were done using the PRIMER v6 computer program[33] with the PERMANOVA+ add-on package[34]. The number of individuals recorded singing any particular MRS type was tallied for each island, yielding a matrix of counts for each of 202 songs in 15 populations, including the Cuvier historical population. These data were square-root transformed and a matrix of Bray-Curtis[35] similarities among populations was calculated. Patterns of relationship were visualised by 2D and 3D non-metric multi-dimensional scaling (MDS)[36] ordination plots (each with 20 random iterative starts). Although the stress was relatively high for the 2D MDS solution (*i.e.*, stress > 0.2), the 3D MDS solution showed the same patterns with a much lower stress value (0.13). Analysis of similarities (ANOSIM)[37] was used to compare the communities of MRS types among the four groups of island populations: ancestral, first-level, second-level or third-level translocations. The index of multivariate dispersion (MVDISP)[38] was used as a measure of the variation in MRS types among islands within the same group.

Heterogeneity in spectral characteristics among the 4 groups of islands having different translocation histories (ancestral, first-level, second-level or third-level) was examined formally using the distance-based test of multivariate dispersions (PERMDISP, 9999 permutations)[39]. Spectral variables (post-transformation and removal of outliers) were then analysed further by averaging their values to the level of whole populations. The averaged data were then standardised to *z*-scores (i.e. normalised) and the relationships among the populations based on the spectral data were examined using principal components analysis (PCA). The relationship between the spectral variables and the MRS type data was then first explored using the "BEST" routine in BIOENV[40], to find the combination of spectral variables whose Euclidean distances have the strongest Spearman rank matrix correlation with the Bray-Curtis similarities obtained from the MRS type data. A more formal model of the MRS type similarity information was then constructed using this chosen subset of spectral variables and distance-based redundancy analysis (dbRDA)[41,42]. This yielded a partitioning of the variation in the MRS type data based on the spectral variables (*via* the DISTLM routine), as well as constrained ordination of the fitted values from the model (*via* the dbRDA routine). Finally, we compared bottleneck levels for each spectral measurement using univariate ANOVA with Tukey's HSD and Bonferroni-adjusted significance levels using Statistica 6 (Statsoft, USA).

2.4 Acknowledgments

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3   Translocation of the North Island saddleback *Philesturnus rufusater* from Tiritiri Matangi Island to Motuihe Island, New Zealand

Health is the capacity of the land for self-renewal. Conservation is our effort to understand and preserve this capacity

-Aldo Leopold 1949 "A Sand County Almanac"

3.1 Summary

Twenty North Island saddleback *Philesturnus rufusater* were translocated from Tiritiri Matangi Island to Motuihe Island in the Hauraki Gulf, New Zealand in August 2005. Post release survival over the first year was high (70%). A minimum of 11 juveniles successfully fledged in the 2005/2006 breeding season bringing the population to a minimum of 25 birds one year after release. Assuming long term success this translocation brings the total number of island populations of North Island saddleback to 13 and contributes to the ongoing ecological restoration of Motuihe Island.

3.2 Background

The North Island saddleback *Philesturnus rufusater* is an endemic New Zealand wattlebird. They are omnivorous forest dwellers, long lived, poor flyers that roost and nest in cavities. By the mid 1900s habitat loss and pervasive predation by introduced mammals had reduced North Island saddleback to a single population of approximately 500 birds on 484-ha Hen Island (Heather & Robertson 1996).

There were three failed attempts to translocate North Island saddleback between 1925 and 1950. The first successful translocation, in 1964 to Whatupuke Island, was facilitated by a greater understanding of the roles of predators at release sites and improved techniques for capturing, holding and transporting sufficient numbers of birds to establish new populations (Lovegrove 1996). Saddleback have become New Zealand's most successfully translocated passerine species and subsequent translocations have increased the total North Island saddleback population to 13 populations on islands free of introduced predators and two

mainland populations at sites with predator proof fences and ongoing trapping and poisoning regimes (Lovegrove 1996; Hooson & Jamieson 2003; Parker 2008).

The conservation status of the North Island saddleback is now secure. However, established island saddleback populations have proved extremely productive, and while adult survival is high, juvenile survival is low due to density dependent factors (Armstrong et al. 2005). This creates opportunities to 'harvest' birds for translocation to new sites, an opportunity further facilitated by the tremendous growth in ecological restoration projects within New Zealand. This case study describes a recent translocation of North Island saddleback to Motuihe Island, using birds from a well-established translocated population on Tiritiri Matangi.

3.3 Action

Motuihe Island: The Motuihe Island Restoration Project is a community led initiative in partnership with the New Zealand Department of Conservation. The project began with an aerial poison operation to eradicate Norway rats *Rattus norvegicus* and house mice *Mus musculus* in 1997 from the 179-ha island. A subsequent aerial poison operation in 2002 was followed up with a combination of shooting, using trained dogs *Canis familiaris*, trapping and poisoning to successfully eradicate introduced European rabbits *Oryctolagus cuniculus* and feral cats *Felis catus*. Intensive re-vegetation was initiated in 2003 to supplement existing native vegetation remnants (which currently cover only about 20 ha) and there have been approximately 165,000 native trees planted up to 2008. There is also ongoing control of exotic plant pests. Saddleback were the first bird species to be translocated to the island, but red crowned kakariki *Cyanoramphus novaezelandiae* have also been recently introduced (May 2008) and there are plans to translocate several other New Zealand endemic bird, reptile and invertebrate species.

Tiritiri Matangi Island: The ecological restoration of 220-ha Tiritiri Matangi Island was initiated in the 1970s. It has involved the planting of 280,000 trees by conservation volunteers and the translocation of 11 bird and three reptile species. In 1984, 24 North Island saddlebacks were introduced. Population growth was rapid and current estimates suggest there are 600-1,000 saddlebacks on the island. The Tiritiri Matangi population had been previously used as a source population for saddleback translocations to Moturoa Island (26 birds, 1997), Mokoia Island (36 birds, 1992) and Karori Wildlife Sanctuary (40 birds, 2002) (Hooson & Jamieson 2003). Given this large population size and no detrimental impacts of

removing birds in this series of earlier translocations, Tiritiri Matangi was again chosen to supply birds for the translocation to Motuihe.

Capture: Three catching teams each of 3-4 people operated during the catching phase 8-10 August 2005. On locating a saddleback territory, a team would erect a 6 or 10 metre mist net. A lure playback system was used consisting of two speakers one on each side and at a distance of approximately 2 m, from the net, connected to a 2-way switch and tape recorder with a selection of saddleback songs. Loud calls were used to attract a saddleback to the vicinity of the net, after which the volume was reduced to small snippets of barely audible calls through the speaker closest to the bird. As the bird approached the net the call volume and frequency were further reduced to tempt the target bird to search for the supposed intruder. When the target bird was close to the net and in a good position to be caught the sound was switched to the opposite speaker enticing the bird to cross the net and be captured. The reaction of birds to lure calls varied between individuals, ranging from a rapid response to no response at all, and some switching between call types was sometimes required to capture the bird. In addition, the position of the net would also sometimes influence outcome with a move of as little as 5-6 m resulting in capture. If a bird was not captured within 20-30 minutes it was usually more productive to move the net to a completely new territory

Captured birds were placed in black cotton bags and then taken to a central processing location. Each bird was banded with a single metal leg band and a unique combination of 1-2 colour plastic bands. The birds were weighed and their tarsus measured to assign sex. Tail and wing measurements were also taken. A small (100 micro litre) blood sample was taken from the brachial wing vein for research investigating the corticosterone response of saddlebacks on different islands. Twenty five captured birds were then housed together for 1-3 days in an aviary approximately 8 m long, 5 m wide and 3.5 m high. The aviary was heavily lined with natural vegetation and leaf litter to provide foraging and roosting opportunities. There were also approximately 30 artificial roost boxes in the aviary and water was supplied in two large (40 cm diameter) dishes. The birds were provided with live mealworms *Tenebrio molitor*, live wax moth *Galleria mellonella* larvae, soaked sultanas, halved oranges and apples, honey water, nectar mix and 'saddleback cake' (Lovegrove & Veitch 1994). The live invertebrates were preferred but small amounts of other food types were eaten.

Translocation: Birds were captured in the aviary with hand nets, reweighed and then placed in groups of five in double ended wooden transfer boxes for transport to Motuihe Island. They were provided with live invertebrates and fruit for the short (approximately 2 hour) boat trip to the island. Water, nectar and honey water mixes were removed prior to transport as the birds can tip or stand in them causing wetting or fouling of their plumage.

Release: On arrival at Motuihe the birds were carried in the wooden transfer boxes to a clear area for a ceremony conducted by the Motuihe Restoration Trust. Here the birds were welcomed by the Trust, representatives from Ngāti Manuhiri and Kawerau a Maki (local *Iwi* or Māori tribes), New Zealand Department of Conservation staff, corporate sponsors and approximately 200 members of the public. They were then taken to the edge of the largest forest patch and released while the crowd looked on. Approximately 150 nesting and 110 roosting boxes were placed throughout bush areas for saddleback prior to release.

Post release monitoring: Thirteen 5-8 hour monitoring trips were conducted between August 2005 and August 2006. Search effort was concentrated on the main bush areas on the island. However, all other potential habitat, including cliff and newly planted areas were checked. Birds were located by listening for calls, systematically searching bush patches and playing pre-recorded saddleback calls to elicit a response from resident saddleback. When a bird was sighted, its location, colour band combination, the presence of other saddleback, and behaviour (pair bonding, courtship, feeding young) was recorded. In addition, both nest and roost boxes were checked for signs of use.

3.4 Consequences

Capture and translocation: Twenty five saddlebacks were captured of which 20, comprising a mixed group of adults (15) and juveniles (5), with 11 male birds and 9 females, were released on Motuihe. The five surplus birds were released at their point of capture on Tiritiri Matangi. No birds died during the capture and holding period. Birds gained an average of 1.36 g (range: -7 – 13 g) in weight in captivity; however, this figure is skewed by the very high weight increases of two individuals (13 and 10 g). If these are excluded from the data set the average weight gain was 0.48 g. There was an oversight in communication between those translocating the birds and the organisers of the release ceremony. This resulted in a minor disruption to the ceremony when the boxes holding the birds were moved to a shaded spot during speeches.

Survival: Fourteen of the 20 birds survived to at least one year after translocation. Two birds were never seen following initial release and four disappeared 6-11 months after being translocated. It is unlikely that any birds escaped detection during post release monitoring as the area of suitable habitat is small (about 20 ha) and saddleback are quite noisy and conspicuous.

Breeding: A minimum of 11 juvenile birds from the 2005/2006 breeding season were detected during post release monitoring. This number is likely to be an underestimate as nestlings were not banded. Two nest boxes were successfully used by one pair. All other nests were undetected and it is likely they were built in the abundant natural cavities on Motuihe. Roost boxes were not heavily used and, again, it is likely that birds were utilising natural cavities.

Conclusions: The survival of translocated saddlebacks on Motuihe (70.0%) in the 12 months following release has been higher than that observed on Cuvier (41.4%), Stanley (45.8%) and Little Barrier (44.0%) Islands and is exceeded only by that observed on Tiritiri Matangi (79.2%) (Lovegrove 1985). In addition, the island has sufficient natural cavities, invertebrate and fruit resources to support successful breeding. Therefore, as long as the pest-free status of Motuihe is maintained it is likely that the population will continue to grow, particularly as recently translocated populations often show increased fecundity and higher juvenile survival due to extended breeding seasons, larger clutches, early breeding and low population density (Craig 1994; Armstrong et al. 2005). The forested area of Motuihe (approximately 20 ha) could support up to 40 pairs of saddleback, thereby allowing significant population expansion. In addition, as the planted areas mature they will also provide important habitat, particularly for young dispersing birds.

A particularly important aspect of this translocation is that it was instigated by a community group, thereby providing valuable advocacy opportunities as well as contributing to ecological restoration, management and scientific goals. Translocations such as this one provide valuable incentive for community based ecological restoration groups to continue and extend their work (Parker 2008).

3.5 Acknowledgments

3.6 REFERENCES

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4 Contemporary cultural evolution of a conspecific recognition signal following serial translocations

...a sexual species' evolution can be directed with all the subtlety of its members' ability to perceive one another

-Will Catton, winner of the 2008 Royal Society of New Zealand Manhire Prize for Creative Science Writing

4.1 Abstract

The divergence of conspecific recognition signals (CRS) amongst isolated populations facilitates the evolution of behavioral barriers to gene flow. The influence of CRS evolution on signal effectiveness in isolated populations can be assessed by testing the salience of changes in CRS from surviving ancestral populations but founder events are rarely detected. The population history of the North Island (NI) saddleback *Philesturnus rufusater* is absolutely known following conservation translocations which increased the number of populations from 1 to 15. With one exception there is no gene flow between these populations. The translocations have generated inter-island divergence of male rhythmical song (MRS), a culturally transmitted CRS. We conducted an experimental test of behavioral discrimination in NI saddlebacks exposed to familiar and unfamiliar MRS and found that responses were significantly stronger for familiar MRS, consistent with a model of contemporary cultural evolution leading to discrimination between geographic song variants. Significantly, this result demonstrates the rapid tempo with which discrimination of CRS might evolve within isolated populations and supports both bottleneck and cultural mutation hypotheses in CRS evolution. The evolutionary implications of contemporary cultural evolution in the production and perception of CRS merit debate on the time frames over which conservation management is evaluated.

4.2 Introduction

The divergence of conspecific recognition signals (CRS) within isolated or partially isolated populations facilitates the development of behavioral barriers to gene flow (Darwin 1871 ;

West-Eberhard 1983; Foster 1999). This process, in concert with ecological factors, genetics and geographical isolation (Edwards et al. 2005; Phillimore et al. 2006; Price 2008) likely influences speciation in birds (Grant and Grant 1997; Slabbekoorn and Smith 2002), particularly the spectacular diversification of oscine passerines (Fitzpatrick 1988) but also suboscine passerines (Seddon and Tobias 2007) and seabirds (Mulard et al. 2009) Bird song plays a critical role in species recognition, mate choice and resource defense, and in many species shows significant geographical variation (Podos and Warren 2007) Consequently, the study of geographical variation in bird song has been critical to our understanding of how diverging CRS can act as reproductive isolating mechanisms (Slabbekoorn and Smith 2002; Podos and Warren 2007; Grant and Grant 2008).

Many studies have shown that birds can distinguish geographical variation in song (Nelson and Soha 2004b; Podos 2007), typically by measuring the response of males to playback of geographical song variants, but also of females (O'Loghlen and Beecher 1999; Hernandez et al. 2009) or pairs (Searcy et al. 1997; Nelson and Soha 2004a; Patten et al. 2004). However, an understanding of how rapidly this perceptual discrimination evolves is lacking (Zuk and Tinghitella 2008). Derryberry (2007) demonstrated that historical white crowned sparrow *Zonotrichia leucophrys* song was a less effective CRS than current song within the same population, supporting the evolution of CRS over time. An alternative approach is to test the salience of changes in communication signals from surviving ancestral populations. This approach has seldom been utilized as founder events are rarely detected (Clegg et al. 2002; Baker et al. 2003; Brunton et al. 2008), and the founder source is often unknown. Subsequently, much of the research investigating geographical variation in bird song has been on continental populations (MacDougall-Shackleton and MacDougall-Shackleton 2001; Searcy et al. 2002; Nelson and Soha 2004b) with varying levels of dispersal and immigration between populations, while few studies (Baker and Jenkins 1987; Lynch and Baker 1993; Baker et al. 2001; Baker et al. 2003; Baker et al. 2006) have examined isolated island populations free of these confounding influences. Therefore, isolated island populations are critical for increasing our understanding of evolutionary processes (Grant and Grant 2008; Price 2008), including the tempo of cultural evolution in bird song and the role it might play in population divergence and speciation.

Conservation translocations provide unique scientific opportunities for studying evolutionary processes as detailed records are often kept of the source, size and age of founder events

(Lovegrove 1996; Parker 2008). In addition, quantifying the long term implications of creating isolated populations through bottleneck events is critical in realizing and evaluating conservation management goals (Lambert et al. 2005). While the impacts of translocations on genetic diversity have been well studied (Lambert et al. 2005; Taylor et al. 2005; Taylor et al. 2007), the effects on behavioral processes are relatively unknown. The North Island (NI) saddleback or tīeke *Philesturnus rufusater* (Holdaway et al. 2001) has been the focus of intense conservation management since 1964 (Lovegrove 1996) and provides an ideal system for studying the time scale of evolution of CRS.

The NI saddleback is an ancient passerine in the family Callaeatidae (Holdaway et al. 2001). Anthropogenic factors reduced the NI saddleback to a single population on Hen Island, New Zealand, by 1910. Although common on that island, the single population was vulnerable to extinction so a series of successful conservation translocations were initiated in 1964 (Lovegrove 1996; Parker 2008). There are currently 15 populations of NI saddleback, including the natural population on Hen Island, a single population on Coppermine Island established through natural dispersal from nearby (c. 150 m) Whatupuke Island (Newman 1980) and 13 populations established through translocation (Figure 6). NI saddleback are weak fliers incapable of crossing water gaps > 250 m (Newman 1980) and with the exception of Coppermine and Whatupuke Islands there is no natural dispersal between any of the populations. Furthermore, DNA analysis indicates that even the population on Coppermine was founded by a small number of founders (Lambert et al. 2005), suggesting that dispersal between the two islands is a rare event.

NI saddleback use male rhythmical song (MRS) for territorial advertisement, defence and mate attraction (Jenkins 1977). MRS is a culturally transmitted CRS that is subject to change through learning errors (Jenkins 1977). Subsequently, the cultural bottlenecks associated with initial translocation and post release cultural mutation appear to have generated inter-island divergence of MRS (K.A. Parker, unpublished data), although genetic effects on song learning and copying accuracy might also have influenced this divergence (Forstmeier et al. 2009). This provides a unique opportunity for studying the evolution of the production and perception of NI saddleback MRS (Parker 2008).

We set out to quantify whether the process of change in MRS between islands has any functional significance as a communication signal for individual NI saddleback populations. The efficacy of CRS have typically been tested using controlled and replicated experimental

Natural population	First level translocation	Second level translocation	Third level translocation	Fourth level translocation
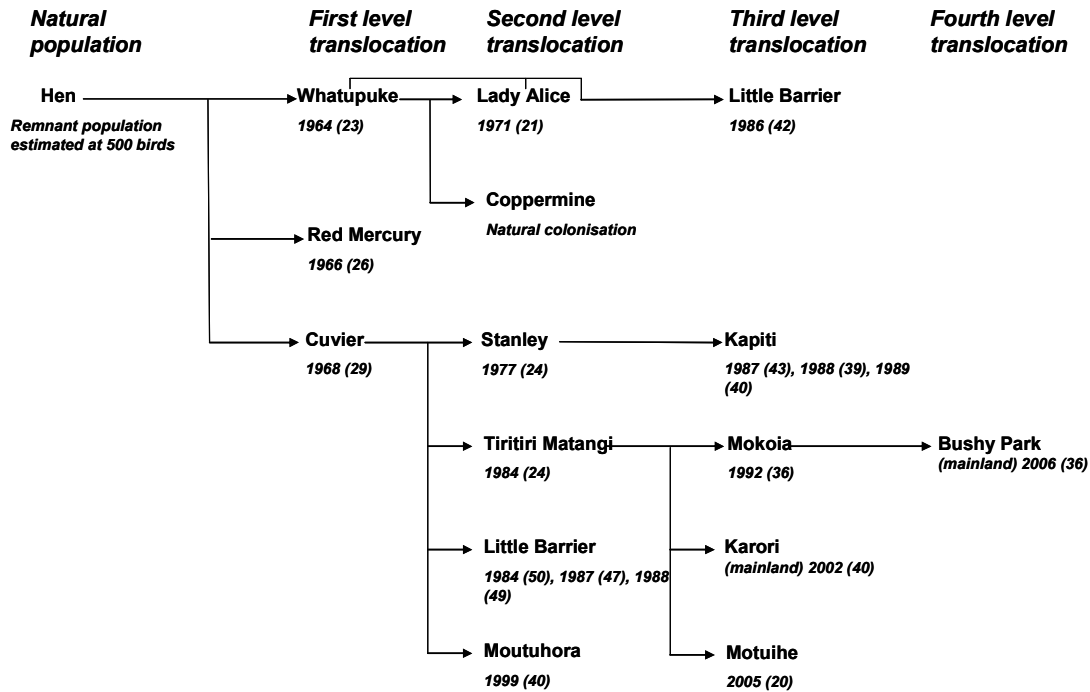
Figure 6. The translocation history of the North Island saddleback showing the years of translocations and the sizes and sources of the founding population (reproduced from Parker (2008)). The population on Little Barrier was founded from three different islands (Whatupuke, Lady Alice and Cuvier) and that on Coppermine is the only example of a natural colonization event. The Kapiti Island population was primarily founded by birds from Stanley Island. However, one bird from Hen, one from Lady Alice or Whatupuke and four birds from Cuvier survived from previous failed translocations (Lovegrove 1996). We used MRS from Hen and Cuvier Islands in playbacks comparing response to familiar MRS on Motuihe Island. We did not use MRS from Tiritiri Matangi Island in our playback experiments, as at least nine founding individuals from Tiritiri Matangi Island survive on Motuihe Island, and subsequently might be familiar with Tiritiri Matangi Island MRS.

playbacks measuring the behavioral reactions (e.g. singing behavior, approach distance, latency to approach and leave the playback area) of resident birds to songs simulating conspecific intruders (Irwin et al. 2001; Patten et al. 2004; Podos 2007). We hypothesized that in playback experiments MRS from different islands will be less effective CRS than local island songs in eliciting territorial behaviors and that the responses of NI saddleback will be greater when exposed to MRS that represent their most recent translocation history. Support for these hypotheses would demonstrate that discrimination of geographical variation in CRS can rapidly (<25 years) establish and might also elucidate the relative roles of translocation induced cultural bottleneck events, or post translocation cultural mutation, in song divergence between isolated island populations. When combined with natural selection pressures

discrimination of CRS might contribute to population divergence, and ultimately to allopatric speciation (Fitzpatrick 1988; Slabbekoorn and Smith 2002; Price 2008). Alternatively, a lack of discrimination would suggest that there is little functional significance in the short term divergence of CRS for NI saddleback, and that it is simply a highly variable behavioral trait under no significant selection pressure for intraspecific function.

Importantly, these alternative scenarios are of interest to both conservation and evolutionary biologists. First, they demonstrate the tempo and mode by which CRS discrimination might evolve, thereby complicating long term conservation management (Anthony and Blumstein 2000; Buchholz 2007; Caro 2007; Angeloni et al. 2008) . Second, they illustrate the potential for conservation management actions, such as translocations, to be potential microevolutionary events, challenging us to look beyond the short time scales (i.e., years or decades) measuring and evaluating conservation actions and to consider the long term implications of conservation biology in an evolutionary context (Ashley et al. 2003; Stockwell et al. 2003).

4.3 Methods

Twenty NI saddlebacks were translocated to Motuihe Island from Tiritiri Matangi Island in 2005 (Parker and Laurence 2008). In November 2008 the population was estimated at 45-50 saddlebacks using the methods described in Parker and Laurence (2008), including 13 territorial pairs and 9 of the original translocated birds.

MRS was recorded using a Sony Hi MD MZ-NH700 mini disc and Sennheiser M66 shotgun microphone. All MRS present in the small (45-50 birds) Motuihe Island NI saddleback population were recorded by one researcher over 1 week in November 2008. Hen and Cuvier Islands were each visited for two 1 week periods in 2005 (Hen, April and August; Cuvier, May) and 2006 (Cuvier, April) by 3-5 researchers who systematically sampled MRS across each island. As controls endemic sympatric grey warbler *Gerygone igata* songs were also recorded on Motuihe Island.

NI saddleback MRS' are easily distinguished both visually and aurally and all recordings were inspected (FFT = 256, Hann window, 5.8 ms, 50% overlap) using Raven 1.2.1 (Cornell Lab of Ornithology, Ithaca, NY, USA) (Figure 7). Subsequently, MRS were randomly
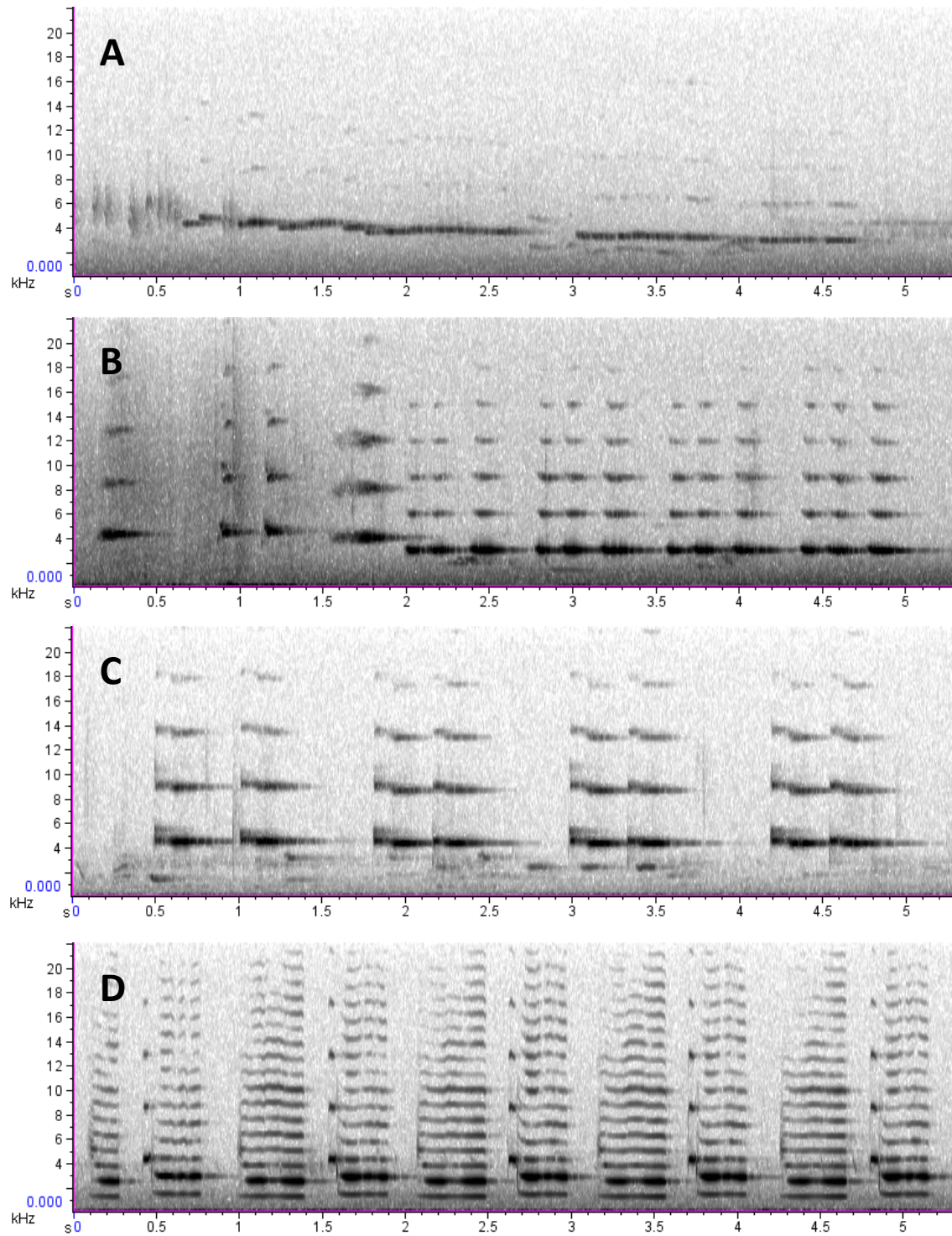
Figure 7. Sonograms showing a single exemplar of each of the four classes of stimuli used in the playback experiments on Motuihe Island; A) control grey warbler; B) Hen Island MRS; C) Cuvier Island MRS; D) Motuihe Island MRS.

selected for playback experiments following comparison and categorization of spectrograms of recordings made on each island.

Playback experiments were conducted on ten pairs of Motuihe Island NI saddleback 1-5 December 2008. NI saddleback form stable long term pair bonds between male and female birds, share and defend a territory year round, forage together, and engage in pair bonding behaviors by giving sexually dimorphic quiet calls (Jenkins 1977). The behavior of test pairs was recorded together for two reasons. First, behavioral responses to territorial intrusions are initiated and maintained by both sexes, although only males give MRS in response to intruders. Second, only 5 of the 10 experimental pairs contained ≥ 1 color banded known-sex birds (NI saddleback can only be reliably sexed in the hand (Jenkins and Veitch 1991) or when giving sexually dimorphic quiet calls (Jenkins 1977)).

Songs from Motuihe Island were considered familiar but we did not play back self and immediately adjacent neighbors. We chose 2 types of unfamiliar songs: MRS recorded from the current Cuvier Island population, which had provided the founding population for Tiritiri Matangi Island (24 birds translocated in 1984), and MRS recorded from the current Hen Island population (the only remaining natural population), which provided the founding population for Cuvier Island (29 birds translocated in 1968) (Figure 6; Parker 2008). Unfamiliar songs from Tiritiri Matangi Island were not used because 5 of the experimental pairs contained at least 1 founder from the 2005 translocation (from Tiritiri Matangi Island to Motuihe Island) that might have been familiar with the MRS.

We recorded all MRS present on Motuihe Island as both the NI saddleback population (45-50 birds, including approximately 13 territorial males) and potential habitat (c. 25 ha) on the island are small. Seven MRS (mean ± SD; 7.6 ± 7.8; range 1-23) were recorded, 5 of which were repeatedly recorded (10.2 ± 7.9; range 3-23) and heard during sampling and playback experiments. NI saddleback MRS are sung throughout the day, year round, particularly in response to conspecific intruders or other territorial disturbances (such as people). Singing behavior peaks at dawn year round but is particularly intense during the breeding season (Murphy 1989). However, 2 of the 7 MRS recorded were only heard on the single occasion on which they were recorded, despite a constant researcher presence over two 5 day periods. Therefore, these 2 MRS were excluded from the playback experiments, due to doubts as to their representativeness as familiar MRS; they might represent adherent or rare MRS that are not in use as territorial signals. Subsequently, 2 exemplars of each of the repeatedly sampled

5 MRS from Motuihe Island, 10 unfamiliar randomly selected MRS from both Hen and Cuvier Islands and 10 different Motuihe Island grey warbler songs were selected to be included in playback sequences for each of the 10 test pairs (3 sequences/pair, 30 sequences total). Sequences consisted of 4 minutes of control songs (1 song/minute, 5 songs total), followed by 8 minutes of silent observation, then 4 minutes of saddleback MRS (1 song/minute, 5 songs total), followed by a further 8 minutes of silent observation. Playback order was randomized, pairs were not tested more than twice in a single day ($\geq$ 4 hours between tests), and immediate neighbours were not consecutively tested. Playbacks were conducted 0630-1930 hours local time using a portable CD player and a Remote Audio Speak Easy Speaker. Playback stimuli were 82-87 dB at 1 m from the speaker, as measured by a sound pressure level meter (Radio Shack 33-2055).

Prior to playback test territories were checked for subject presence (NI saddleback pair within 80m of the speaker). Five minutes of pre-playback observations confirmed the behavioral status of test pairs; playbacks were delayed if territorial confrontations were occurring. During pre-playback, playbacks and observation periods the location of the test pairs relative to the speaker, MRS/minute and chatter calls/minute were recorded by a single observer 12 m from the speaker. Distance was estimated at 0-4 m from the speaker, 4-8 m, 8-12 m, 12-20 m and >20 m with flagging tape on vegetation to 12 m to assist estimation.

The responses of test pairs during experiments were grouped into a single 12 minute period. Responses compared between control and test stimuli included: MRS/minute (male birds only), chatter calls/minute, the closest approach to the speaker (the median measure for each distance category, i.e. an approach to 0-4m was analyzed as an approach to 2m. Approaches >20m were analyzed as 21m), the latency to approach to ≤12 m of the speaker (minutes from the start), and the latency to leave the playback area (proportion of the 12 minute experimental period within 12 m of the speaker). The latter two responses were restricted to ≤ 12 m of the speaker due to difficulties in detecting birds in dense vegetation at 12-20 m. A more intense response to playback by test pairs was considered to be increased calling behaviour (MRS from males only, chatter calls from both sexes), a closer approach to the speaker, a short latency to approach and a longer latency to leave the playback area

Kroodsma (2001) suggested that to avoid pseudoreplication the number of test stimuli used should be treated as the sample size rather than the number of test subjects. In our experiments we used a random sample of 10 different MRS from each of the 2 unfamiliar

islands. Ideally, we would have also used 10 different familiar MRS from Motuihe but as described above we are confident that a maximum of 7 MRS types exist on Motuihe, 2 of which were only recorded and heard on one occasion and might not actually be representative MRS. In any case, the 5 familiar MRS used in the experiments represent at least 70% of the familiar song diversity on Motuihe. We also controlled for pseudoreplication at the level of the recording or rendition of an MRS type by any particular individual by using 2 different exemplars of each of the 5 Motuihe MRS.

Each pair's responses to control songs were compared to familiar MRS, and to unfamiliar MRS (average pair responses to Cuvier and Hen MRS), using Wilcoxon's Matched Pairs tests. We also used the same test to check for order effects by comparing behavior one minute immediately before and after the control songs. We also checked if responses to playback were from a single bird or both members of the pair using Friedman's analysis of variance (ANOVA).

Each test pair's behavioral responses to Hen, Cuvier and Motuihe MRS were then compared using Friedman's analysis of variance (ANOVA) and Kendall's coefficient of concordance. NI saddlebacks do not give a singular response to behavioral intrusions and strategies vary between individuals. Therefore, as a final test we conducted a Principal Components Analysis (PCA) using all the behavioral responses to construct the principal components and repeated the above statistical comparisons. Alpha was set at 0.05 for all statistical tests.

4.4 Results

PC1 explained 65% of the variation in raw response parameters and loadings were consistent with a closer approach to playback of familiar Motuihe Island MRS and unfamiliar Cuvier Island MRS, approaching the playback area more rapidly, and a greater latency to leave the playback area when compared to unfamiliar Hen Island MRS. PC2 only explained 17% of the variation in raw response parameters (Table 5) and is not included in further analyses.

The behavioral responses and PC1 scores of test pairs to familiar and unfamiliar MRS were all significantly greater than those given to control songs (Table 6; Figure 8a-c). There were no approaches to the playback area and no significant order effects when calling behavior (mean ± SD; MRS before = 0.13 ± 0.17; MRS after = 0.03 ± 0.11; Wilcoxon $Z = 1.60$, $N = 10$, $P = 0.11$; chatter calls before = 0.07 ± 0.14; chatter calls after = 0.03 ± 0.11; $Z = 0.53$, N

Table 5. Principal component factor loadings for the analysis of behavioral responses of Motuihe Island saddleback to playback of grey warbler songs (control) and Motuihe, Cuvier and Hen Island MRS. The highest values for each PC factor are shown in bold and the percentage of variation across all responses explained by each PC factor is shown in parentheses.

Behavioral response	PC1 (65%)	PC2 (17%)
MRS	0.56	**-0.66**
Chatter calls	0.56	**0.65**
Approach distance	**-0.93**	-0.07
Latency to approach	**-0.95**	0.00
Latency to leave	**0.93**	-0.06

Table 6. Descriptive statistics (means ± SD) and test statistics (Wilcoxon's matched pairs test) for response variables and PC1 comparing playbacks of control song (grey warbler) with playbacks of familiar MRS (Motuihe Island) and unfamiliar MRS (Cuvier and Hen Island) on Motuihe Island.

| | Stimulus | | | Comparison | | | |
| | | | | Control/Familiar | | Control/Unfamiliar | |
Response	Control (N=10)	Familiar (N=10)	Unfamiliar (N=10)	Wilcoxon Z score	P value	Wilcoxon Z score	P value
MRS	2.00 ± 1.79	10.7 ± 7.51	2.1 ± 4.55	2.67	0.01	21.50	0.01
Chatter calls	2.43 ± 1.21	7.9 ± 8.08	6.3 ± 5.90	2.11	0.04	2.19	0.02
Approach distance	16.90 ± 3.17	3.4 ± 4.43	8.7 ± 8.52	2.80	0.01	2.41	0.01
Latency approach	11.10 ± 1.69	2.9 ± 3.24	6.4 ± 4.62	2.70	0.01	2.24	0.01
Latency leave	0.04 ± 0.06	0.69 ± 0.40	0.38 ± 0.38	2.70	0.01	2.52	0.01
PC1	-0.93 ± 0.24	0.92 ± 0.70	0.01 ± 0.87	2.70	0.01	2.41	0.02

$= 10$, $P = 0.60$) and closest approach to the speaker (approach before $= 12.30 \pm 8.96$m; approach after $= 4.20 \pm 8.85$m; $Z = 1.86$, $N = 10$, $P = 0.06$) were compared one minute before and after control playbacks. With few exceptions the response to playback was from the territorial pair rather than a single bird (Motuihe $= 1.9 \pm 0.32$; Cuvier $= 1.8 \pm 0.42$; Hen $= 1.8 \pm 0.42$) and there was no significant difference in the number of birds (the territorial pair versus a single bird) responding to calls from Motuihe, Cuvier or Hen Islands (Friedman $\chi^2 = 1.00$, $N = 10$, $P = 0.61$).

There were significant differences in the number of MRS given by test males in response to playback of familiar and unfamiliar MRS (Friedman χ^2 = 11.73, N = 10, P < 0.01) (Figure 8a). A *post hoc* nonparametric multiple comparison (Zarr 1999) showed that males gave significantly more MRS in response to familiar Motuihe Island MRS than to unfamiliar Cuvier Island MRS (q = 3.95, P < 0.05) or Hen Island MRS (q = 4.11, P < 0.05) but there was no significant difference between the responses to unfamiliar Cuvier and Hen MRS (q = 0.16, P > 0.05). There were no significant differences in latency to approach the speaker (χ^2 = 5.89, N = 10, P = 0.05), chatter call rates (χ^2 = 0.65, N = 10, P = 0.72), approach distance (χ^2 = 3.26, N = 10, P = 0.20) or latency to leave the playback area (χ^2 = 3.30, N = 10, P = 0.31).

Responses by test pairs to familiar and unfamiliar MRS differed significantly according to PC1 (χ^2 = 7.40, N = 10, P = 0.03). A *post hoc* nonparametric multiple comparison (Zarr 1999) showed significantly more responses to familiar Motuihe Island MRS when compared to unfamiliar Hen Island MRS (q = 3.48, P < 0.05). There were no significant differences between familiar Motuihe Island MRS and unfamiliar Cuvier Island MRS (q = 3.16, P > 0.05), or between unfamiliar Cuvier Island MRS and unfamiliar Hen Island MRS (q = 0.32, P > 0.05) (Figure 8a-c).
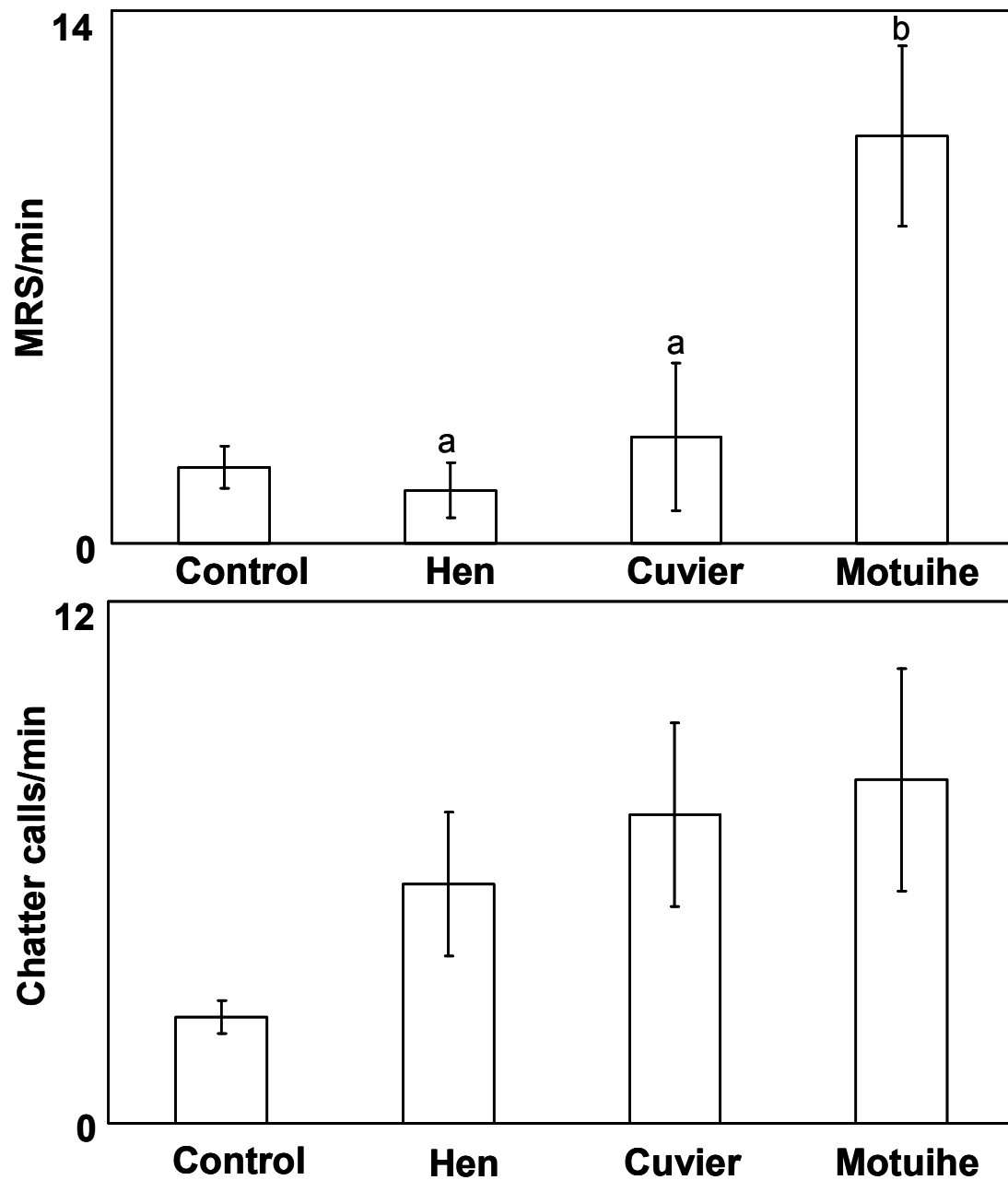
Figure 8a. Behavioral responses of Motuihe NI saddleback to playback of control grey warbler song, familiar Motuihe Island MRS and unfamiliar Cuvier and Hen Island MRS. Means ± SE are shown. $N = 10$ pairs. The bars labeled a and b on the MRS graph are significantly different ($P < 0.05$) but there were no significant difference in the chatter calls following playback.
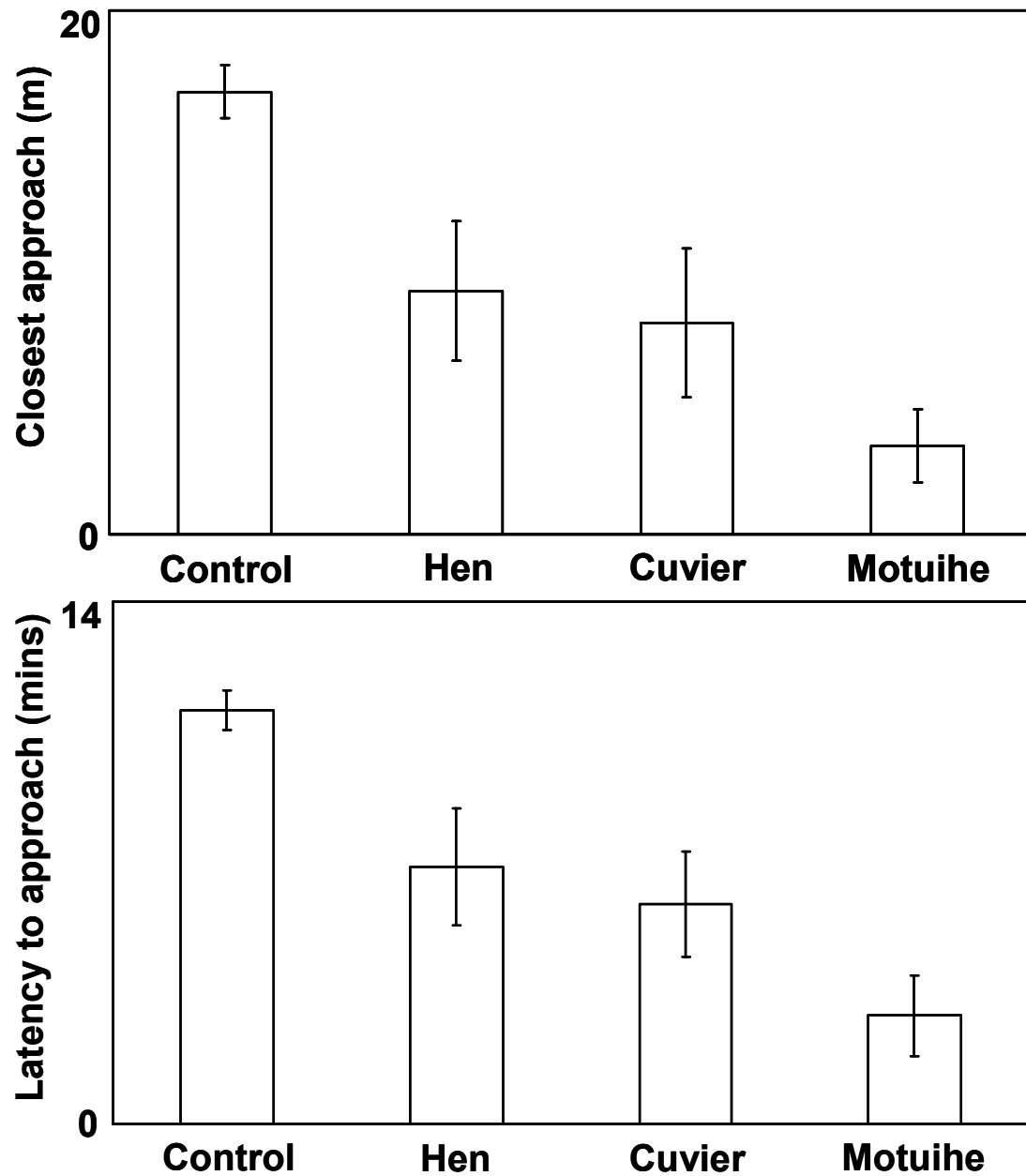
Figure 8b. Behavioral responses of Motuihe NI saddleback to playback of control grey warbler song, familiar Motuihe Island MRS and unfamiliar Cuvier and Hen Island MRS. Means ± SE are shown. $N = 10$ pairs. There were no significant differences in closest approach or latency to approach.
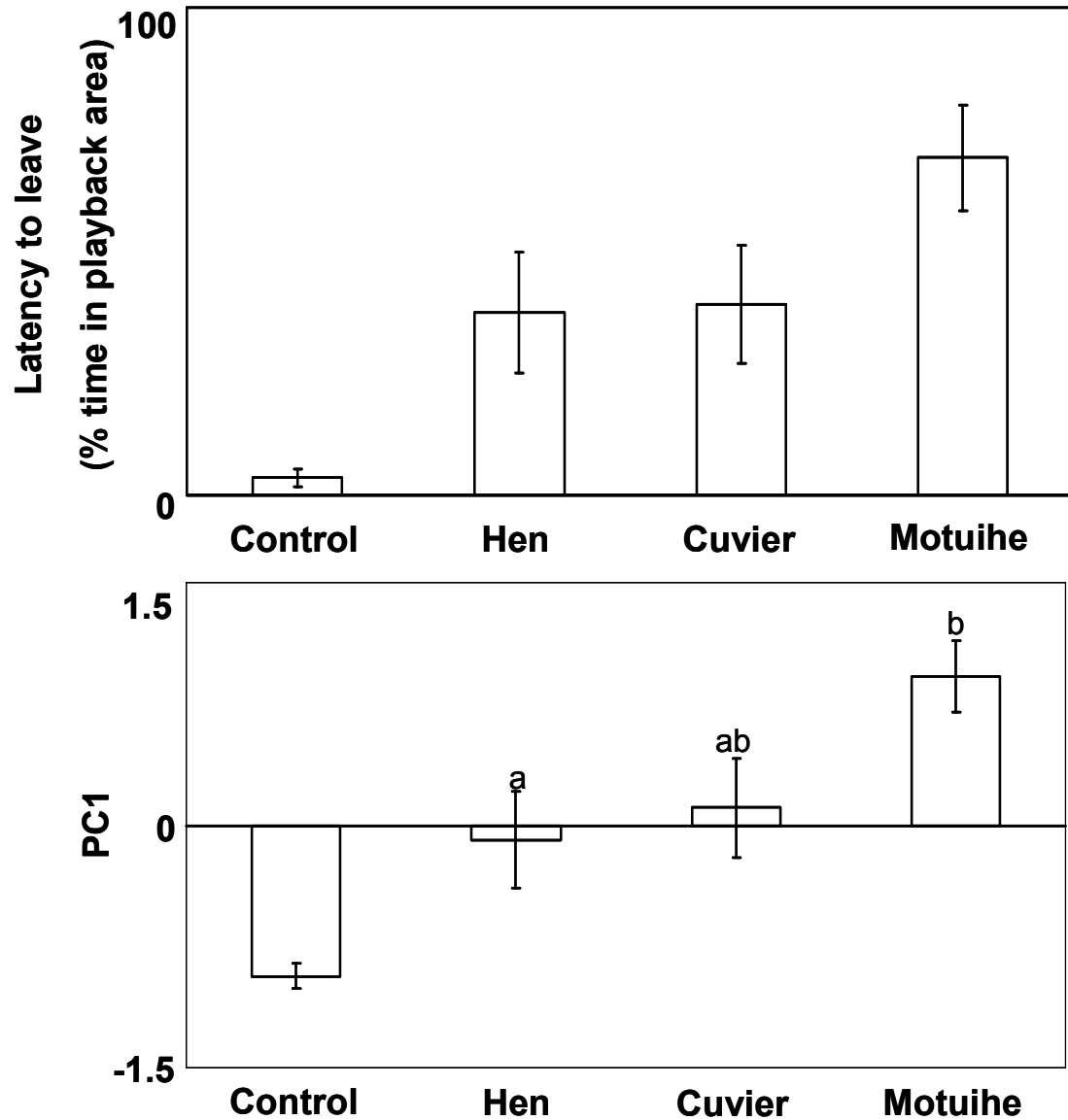
Figure 8c. Behavioral response and PC1 of Motuihe NI saddleback to playback of control grey warbler song, familiar Motuihe Island MRS and unfamiliar Cuvier and Hen Island MRS. Means ± SE are shown. $N = 10$ pairs. The bars labeled a and b on the PC1 graph are significantly different ($P < 0.05$) but there were no significant differences in latency to leave following playback.

4.5 Discussion

Our playback experiments of conspecific song types are consistent with the rapid establishment of behavioral discrimination between geographic song variants by NI saddleback pairs following serial translocations. Critically, these results support the

prediction of the contemporary evolution hypothesis that familiar MRS is a more effective CRS than unfamiliar MRS. First, territorial NI saddleback pairs showed consistent behavioral discrimination in all responses separately and the combined first principal coordinate when control heterospecific grey warbler songs was compared to that given to either unfamiliar or familiar conspecific MRS (Table 5; Table 6). Second, the first principal coordinate showed significant discrimination of familiar Motuihe MRS over unfamiliar Hen to be consistent with a shorter latency to approach the playback area, a closer approach to the playback speaker, and a greater latency to leave the playback area (Table 6; Figure 8a-c).

When the different behavioral responses to playbacks are examined separately the number of MRS given by males in response to unfamiliar Hen and Cuvier MRS was significantly less when compared to familiar Motuihe MRS, and post hoc tests showed the greatest response to familiar Motuihe MRS. There were no statistically significant differences in the other behaviors or with increasing distance from the ancestral population in response to playback of MRS.

While many studies demonstrate discriminatory behaviors in playbacks of familiar versus unfamiliar song (Searcy et al. 1997; Nelson and Soha 2004b; Patten et al. 2004; Podos 2007), we know of few wild population studies (Derryberry 2007) with such detailed information on the pace (<25 years) at which this discrimination has evolved. Distinguishing the causal mechanisms that have led to the divergence of MRS between islands is more difficult. We have demonstrated that MRS from different islands is less recognized as a conspecific territorial signal. This might be explained by two hypotheses. Under a cultural bottleneck hypothesis when NI saddleback are translocated to a new island there would be a loss of song diversity in translocated populations relative to the severity of the bottleneck, and a subsequent loss of response to MRSs absent within translocated populations. Alternatively, a cultural mutation hypothesis would predict that the social transmission of MRS is subject to a high rate of learning errors, with a subsequent increase in cultural mutations (Jenkins 1977) and rapid changes in song diversity both within and between populations. Future work focusing on reciprocal experiments in ancestral populations might fully resolve these two hypotheses, but our results provide an initial insight into these alternatives. If cultural mutation has led to divergence Motuihe Island NI saddleback pairs should treat both Hen Island and Cuvier Island MRS as unfamiliar when responses are compared to familiar Motuihe MRS. In contrast, if a cultural bottleneck has led to divergence then the response to

unfamiliar Cuvier and familiar Motuihe should have been more similar than to the unfamiliar ancestral MRS from Hen Island, particularly given that the initial bottleneck was severe (Figure 6) relative to subsequent bottlenecks, where cultural diversity would have already been reduced. These hypotheses are not mutually exclusive and our findings offer support to both. The behavior of test pairs, as represented by PC1 support the bottleneck hypothesis. However, when examined individually the behaviour of males, as measured by MRS call rates in response to playback, support the cultural mutation hypothesis. A bioacoustic analysis of MRS from all islands is beyond the scope of the analyses presented here. However, initial results (K.A. Parker, unpublished data) also support both hypotheses and we suspect the contribution of each will be relative to individual island history, that is, how far any island is removed from the ancestral population on Hen Island (Figure 6).

We have demonstrated that unfamiliar MRS is a less recognized CRS than familiar MRS. But despite rapid and sometimes aggressive responses in our experiments, with one bird perching on top of and pecking a speaker for > 1 minute during experimental playbacks, our data do not inform us as to the relative strengths of individual MRS in holding a territory. However, given experimental evidence from other species, including song sparrows *Melospiza melodia* (Nowicki et al. 1998), and the primary role of MRS for territorial defense and advertisement in NI saddleback (Jenkins 1977) it is a reasonable assumption that playback responses might correlate with territory holding behavior. Male removal and replacement with a speaker might elucidate this relationship. However, Falls (1988) suggests that speaker replacement experiments might be most effective with visually inconspicuous species, and in NI saddleback delivery of MRS is loud, conspicuous, close to (< 10 m), and within sight of any intruder and accompanied by stereotyped body posturing. Therefore, speaker replacement experiments might also require the use of a complex "robotic" model NI saddleback after Searcy (2008).

The heterospecific controls used in these experiments anchor the directionality of behavioral responses, but it is also possible that responses to variation in conspecific and heterospecific social signals are not linear, but rather curvilinear. Under this scenario NI saddleback might possess a template for CRS that prompts a strong response to intermediate signals but poor responses to both best and poor matches, in a similar manner to Bateson's (1982) work on Japanese quail *Coturnix coturnix japonica* which showed a preference for first cousins (intermediate phenotype match) over siblings (best phenotype match) and unrelated birds

(poor phenotype match) in choice tests. Alternatively, there might be a curvilinear response to low, medium or high performance level of the signal, as hypothesized by de Kort (2008).

The tempo of cultural evolution of NI saddleback song can clearly occur over time spans (<25 years) consistent with a model of contemporary cultural evolution. Given this short time frame, we consider that the NI saddleback currently fits within the early stages of Slabbekoorn and Smith's (2002) model of acoustic divergence with song learning. Under this model NI saddleback share a common acoustic space, in that all populations remain physiologically capable of producing all the MRS patterns present within other populations and the realized diversity currently observed within individual populations represents phenotypic variation in MRS.

The relationship between gene flow and geographic variation in song in NI saddlebacks in particular, and in songbirds in general, remains unclear. While some studies have found a correlation between song dialects and restricted gene flow (Irwin et al. 2001; MacDougall-Shackleton and MacDougall-Shackleton 2001) others have not (Soha et al. 2004; Nicholls et al. 2006; Leader et al. 2008). NI saddlebacks are an important model because with the exception of two of the 13 islands there is no natural dispersal between any of the populations. Dispersal between populations could only be emulated via translocations and might be desirable for genetic management to increase heterozygosity (Lambert et al. 2005) but it is not clear what the effect of divergence in the behavioral recognition of and responses to CRS will be if birds are translocated between established populations. In a population of the closely related NI kokako *Callaeas cinerea wilsoni* (Ewen et al. 2006) established on Kapiti Island through translocations from several distant geographic origins, birds mated assortively with individuals from the same source population (Rowe and Bell 2007) but this behavioral mating pattern disappeared with the first island hatched progeny. In parallel, one practical implication of our work is the recommendation that future translocations of NI saddleback should proceed simultaneously from multiple source populations.

We speculate that translocating between established populations will be challenging but only well monitored experimental translocations will elucidate the role of familiar MRS and territory holding ability. However, while adult NI saddleback are capable of learning a new MRS Jenkins (1977) suggested this is infrequent. Young birds moving into a territory typically perfect existing MRS in weeks or months, possibly learnt whilst non-territorial floaters, but adults might take up to one year. It is not clear if there would be a cost to a male

NI saddleback singing an unfamiliar MRS or learning a new MRS however there is limited evidence from species such as song sparrows that singing a familiar song might be advantageous to territory holding ability (Beecher et al. 2000a; Beecher et al. 2000b; Wilson et al. 2000). The NI saddleback social system is based on territorial neighbors sharing the same MRS, and the rare examples of non-conforming birds seem to be temporary phenomenon, with birds either learning the predominant MRS or shifting territories (Jenkins 1977). Density-dependent survival is evident in established NI saddleback populations, territorial vacancies are rare and rapidly filled by local recruits (Armstrong et al. 2005) and birds that can quickly conform might have an advantage. Therefore, reciprocal translocations of adult birds (possibly $\geq$ 100) might be required to create territorial openings at the respective sites. Several studies in other songbirds show that female birds also prefer familiar song over unfamiliar song (Searcy et al. 1997; O'Loghlen and Beecher 1999; Patten et al. 2004; Hernandez et al. 2009) but such studies are rarer, and exceptions have been reported (Nelson and Soha 2004a). Translocation of eggs or chicks for local song tutoring might also be viable management options but will be expensive, logistically and ethically challenging.

The evolutionary impact of geographical variation in song ultimately depends on patterns of signal perception and discrimination (Podos 2007) and existing populations of NI saddleback are likely to continue to diverge acoustically with the existing discriminatory behaviors further reinforced. Over time NI saddleback might proceed to the latter stages of Slabbekoorn and Smith's (2002) model of acoustic divergence with learning, where song will become a reliable population marker following divergence of potential acoustic variation between island populations. This process might proceed relatively quickly given the small founder populations (generally 20-40 birds) and small offshore and mainland islands in which they can exist (135-3000 ha) (Grant and Grant 1996; Lovegrove 1996). Ultimately, the evolution of behavior, along with genetic drift and ecological factors prompting natural selection, might lead to an allopatric model of speciation within translocated populations of NI saddleback. An oft-stated goal of conservation biology is to preserve evolutionary potential but conservation practice can also direct evolutionary trajectories (Stockwell et al. 2003). Subsequently, there is increasing recognition of the impact of management actions as potential micro-evolutionary events leading to a call for evolutionary enlightened management (Ashley et al. 2003), particularly where conservation mediated changes in behavior might lead to an eventual reduction in the effective population size of managed species (Anthony and Blumstein 2000). This is clearly a long term phenomenon in a world

of pressing short term conservation problems. Nonetheless, it warrants debate as to the management significance of long term divergence and also provides a unique research opportunity; that is while the benefits of long term studies of ecology are acknowledged, long term studies of evolution are less recognized (Grant and Grant 2002).

Our study demonstrates how behavioral research can contribute to a short term understanding of the consequences of conservation management on perceptual selectivity of song variants and a long term understanding of the evolutionary potential of conservation management of individuals from diverse source populations (Anthony and Blumstein 2000; Buchholz 2007; Caro 2007; Angeloni et al. 2008). Speciation in birds proceeds with the evolution of behavioral barriers to gene flow (Grant and Grant 1997) and evolution is a process, not an event (Grant and Grant 2008). However, with current conservation management programs, evolution might prove to be a process that proceeds at the scale of each translocation event.

4.6 Acknowledgments

4.7 References

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5 Defining species limits in the New Zealand saddleback *Philesturnus spp*. using vocalisations, morphology and mtDNA

As the late Professor A.J. Cain remarked: 'An animal is not in the best position to demonstrate the function of its characteristics when lying on its back in a museum drawer.' How much more true this may be when the creature is represented only by molecules in a test tube.

-Ian Newton 2003 "The speciation and biogeography of birds"

5.1 Abstract

Appropriate and defendable definitions of species limits are critical for both scientific study and conservation management of biodiversity. Advances in the analysis of sound and genetic data have proven invaluable in assessing species limits particularly in birds that show considerable geographic variation in these traits. Many birds are currently defined as subspecies on the basis of this variation. However, it has been suggested that following close examination many of these subspecies will be recognised as good species. North Island (NI) (*Philesturnus carunculatus rufusater*) and South Island (SI) saddleback (*P. c. carunculatus*) show significant differences in plumage and differing vulnerabilities to introduced predators. Here, we formally compare geographical variation in NI and SI saddleback vocalisations, morphology and mtDNA and assess these differences relative to their current subspecies status. NI and SI saddlebacks show highly significant differences in both male specific song and loud chatter calls, the two primary vocalisations used by saddlebacks. Furthermore, a discriminant analysis successfully allocated 90% of observations to the correct group based on significantly larger tarsus, weight and wing measurements in SI saddlebacks when compared to NI saddlebacks. Finally molecular data show ~5% sequence divergence in mtDNA between the two groups. The plumage differences alone are sufficient to assign full species status under the criteria of Helbig et al. (2002) and the vocalisation and mtDNA data presented here support this elevation. However, we suggest additional analyses of museum specimens from extinct mainland populations of both groups may further elucidate the taxonomic status of NZ saddleback.

5.2 Introduction

There are both practical and theoretical reasons for defining and classifying species. Subsequently, the debate as to what constitutes a species is long running, often rancorous and, tellingly, shows no sign of conciliation (Coyne and Orr, 2004; Price, 2008; Townsend Peterson, 1998). However, scientific progress and conservation management rely on objective and accurate definitions, despite the inherent challenges (Funk et al., 2002; Moritz, 2002). The biological species concept (BSC) (Coyne and Orr, 2004; Mayr, 1969; Price, 2008) has long been dominant in ornithology but many viable alternatives have been proposed including the phylogenetic species concept (PSC) (Cracraft, 1983; McKitrick and Zink, 1988), the evolutionary species concept (ESC) (Weins, 2004; Wiley, 1978) and the general lineage concept (GLC) (de Queiroz, 1998, 1999).

Helbig et al. (2002) precisely stated that differences between concepts are primarily a matter of emphasis but suggested that the GLC encompassed many biologists views of a species. They determined that species are population lineages that maintain their integrity in space and time relative to other lineages, are diagnosably different, reproductively isolated, share a common mate recognition and fertilization system and have their own independent evolutionary histories (Helbig et al., 2002). Helbig et al. (2002) acknowledged that species concepts disagree in predictions about the outcome of future contact between species and proceeded to delimit and define species based on two criteria; 1) are the taxa diagnosable, e.g. on the basis of plumage, vocalisations, morphology or DNA sequences? 2) are they likely to retain their genetic and phenotypic integrity in the future?

Recent ornithological taxonomy within New Zealand (NZ) has largely adhered to the BSC (Heather and Robertson, 1996; Higgins et al., 2006; Oliver, 1955), with particular use of the subspecies concept to define geographically distinct groups between the main islands. However, the subspecies category itself is controversial (Rising, 2007; Winker et al., 2007). This is understandable particularly in continental systems where overlapping distributions of clinal species complicate rigorous definition of species limits (Cicero and Johnson, 2006; Patten and Unitt, 2002). But the evolutionary processes influencing island species are often quite different, the potential for rapid change much more apparent (Rising, 2007) and the distinction between species and subspecies, at least in comparison to continental systems, perhaps more definitive. Holdaway et al. (2001) recognise this distinction in a recent revision

of the breeding birds of NZ and argue for a reclassification of many NZ subspecies as good species.

The New Zealand saddleback (*Philesturnus carunculatus*) is an ancient passerine in the family Callaeatidae (Holdaway et al., 2001). The saddleback was first described by Māori who called it either tīeke, an onomatopoeic name based on the birds' loud call, or tiaki meaning to guard or keep watch (Orbell, 2003). The first European record of the South Island (SI) saddleback (*P. c. carunculatus*) was in Queen Charlotte Sound in January 1770 while the North Island (NI) saddleback (*P. c. rufusater*) was recorded in the Bay of Islands in 1772 (Oliver, 1955; Watola, 2007). The two forms have generally been considered subspecific (Heather and Robertson, 1996; Higgins et al., 2006; Oliver, 1955; Turbott, 1990; Williams, 1976) but Holdaway et al. (2001) cite earlier work, distinct plumage differences and disparity in body size in calling for full species status for the NI and SI birds. Specifically, immature and juvenile SI saddlebacks have a brown plumage in contrast to the mostly black plumage of adult SI birds whereas immature and juvenile NI saddlebacks closely resemble adult NI birds (Higgins et al., 2006). Adult NI birds have a thin yellowish band at the front of the mantle that is absent in SI birds and the SI birds are larger than NI birds. There also appears to be striking differences in vulnerability to introduced predators with NI saddleback both persisting and establishing in the presence of the non-native kiore or Polynesian rat (*Rattus exulans*) while the SI saddleback cannot (Hooson and Jamieson, 2003; Lovegrove, 1996). Distinct differences in vocalisations are also apparent.

Advances in analytical methods, particularly of vocalisations and genetics, allow for careful appraisal of avian subspecies with some likely being reclassified as good species (Rising, 2007; Townsend Peterson, 1998). Here, we formally compare NI and SI saddleback vocalisations, morphology and mtDNA and assess these differences relative to the current subspecific status of these two taxonomic groups using the criteria described in Helbig et al. (2002) for establishing species limits.

5.3 Methods

Our NI saddleback data, particularly the vocalisation data, are drawn from all existing NI populations whereas the SI vocalisation and morphological data are from a single population on Ulva Island, and the mtDNA from Big Island, two of 19 SI saddleback populations (Hooson and Jamieson, 2003; Masuda et al., 2010). If the SI data we present are significantly

different to all NI populations then we think this comparison is sufficient for assessing distinctiveness between the two groups. Furthermore, for the purposes of these analyses we are only interested in differences between the NI and SI populations; variation within the NI saddleback data are discussed elsewhere (Chapter 2).

5.3.1 Vocalisation recording and definition

NI saddleback vocalisations were sampled by 1-4 researchers on each of the 13 established island populations (Hooson and Jamieson, 2003; Parker, 2008) on 1-4 trips of 5-15 days between December 2004 and November 2008. Vocalisations were recorded using Sony Hi MD MZ-NH700 mini discs, Sennheiser K6 recording modules, M66 microphones and Rycote Softy wind guards mounted on Rycote pistol grips. They were defined as loud chatter calls (given by both sexes), sexually dimorphic quiet calls and male rhythmical songs (MRS) (Jenkins, 1977). SI saddleback vocalisations were recorded on Ulva Island from October to December 2004 using a Sony TCD-D8 digital audio tape recorder, Sennheiser MKH 815T microphone and Rycote windshield following the method of Ludwig and Jamieson (2007). NI saddleback calls were downloaded and converted to WAV. format using Sony SonicStage software (Sony Corporation 2001-2007) with 16 bit sampling precision and a 44 kHz sampling rate. SI saddleback vocalisations were also converted to the same format (Ludwig and Jamieson, 2007). SI saddleback vocalisations were defined as chatter calls, quiet calls and male specific phrases (Ludwig and Jamieson, 2007). Subsequent analysis was restricted to a comparison of NI saddleback MRS and the analogous SI saddleback male specific song and a separate comparison of NI and SI saddleback chatter calls.

NI and SI saddleback vocalisations are easily distinguished both visually and aurally by researchers (Ludwig and Jamieson, 2007; Parker et al., 2010) and all recordings were inspected (FFT = 256, Hann window, 5.8 ms, 50% overlap) and categorised as chatter calls, NI saddleback MRS or SI saddleback male specific phrases using Raven 1.2.1 (Cornell Lab of Ornithology, Ithaca, NY, USA). NI saddleback MRS consists of highly stereotyped repeated phrases sung by male birds in territorial displays (Jenkins, 1977) (Figure 9a). SI saddlebacks sing male specific phrases comparable to NI saddleback MRS (Figure 9b) but do not show the high degree of stereotypy observed in NI saddleback and instead deliver songs using partial and full phrases in a mixed mode and random order (Ludwig and Jamieson, 2007). Given that both NI and SI birds use phrases to form their songs, albeit in

different ways, we used a single phrase from each male song as the unit of song comparison (Figure 9a - 1b).

NI and SI chatter call are similar (Figure 9c-d) both consisting of repeated phrases delivered in widely varying lengths depending on the social context of their delivery (Jenkins, 1977; Ludwig and Jamieson, 2007). Therefore, for our unit of comparison we measured three consecutive chatter phrases within a single chatter call (Figure 9c-d).

Spectral measurements of individual NI and SI phrases were taken in Sound Analysis Pro (Tchernichovski et al., 2004) (Version 1.02) using the manual segmentation procedure with a 200 Hz high pass filter (data window = 9.27 ms, advance window = 1.36 ms, spectrum range = 11 180 Hz, Fast Fourier Transform window = 1024 samples, tapers = 2, bandwidth parameter = 1.5). The following spectral variables were used for analysis (see Tchernichovski (2004) for definitions of the spectral variables): duration, mean amplitude modulation, and both the mean and the variance of pitch, entropy, frequency modulation, goodness of pitch and mean frequency. Two data sets were created from this procedure, one consisting of NI saddleback MRS and SI saddleback male specific phrases and one consisting of NI and SI chatter calls.

Spectral variables were examined for normality and the following variables were transformed to $\ln(x)+0.01$ to remove skewness for all male songs and chatter calls: duration, mean pitch, variance in pitch and variance in goodness of pitch. The following variables were transformed for analysis of male songs but not for chatter calls; mean goodness of pitch, mean frequency and variance in entropy. Extreme outliers were examined both visually and aurally for corrupting background sounds and removed (1.8%, N = 6) from the chatter call data set prior to subsequent analysis.

Heterogeneity in spectral characteristics of male songs and chatter calls among NI saddleback populations and Ulva Island SI saddleback was examined using the distance-based test of multivariate dispersions (PERMDISP, 9999 permutations) (Anderson, 2006) following standardisation to z-scores (i.e. normalisation). Spectral variables were further analysed using island population averages and examining the relationships among these populations using principal components analysis (PCA). Finally, we compared spectral variables from each data set using univariate one way ANOVA with Tukey's HSD and Bonferroni-adjusted significance levels.
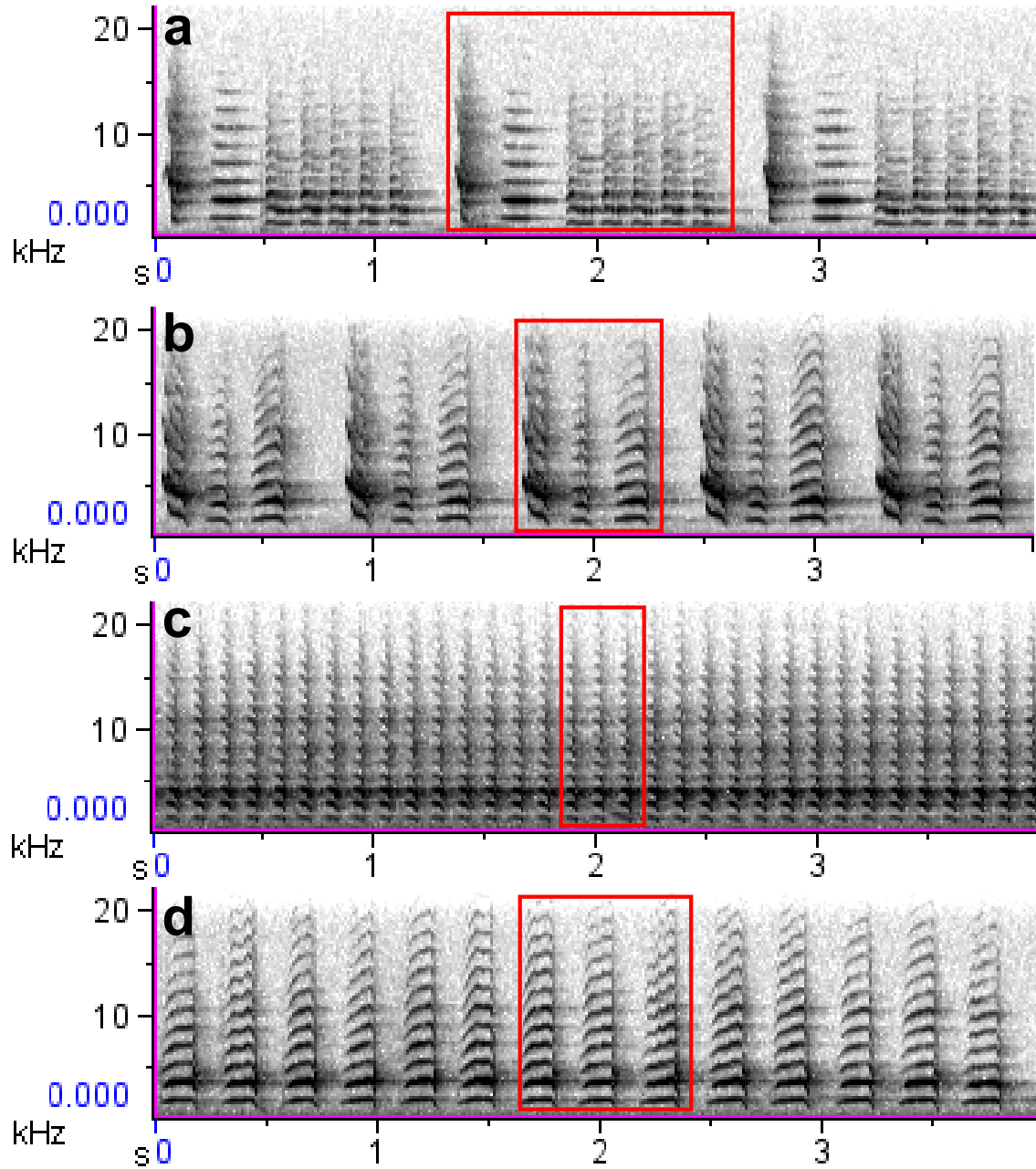
Figure 9. Spectrograms of a) a NI saddleback MRS from Mokoia Island b) a SI saddleback male specific song from Ulva Island c) a NI saddleback chatter call from Moutuhora Island and d) a SI saddleback chatter call from Ulva Island. The red outlines show a single phrase (a, b) and three consecutive chatter phrases (c, d) as measured for spectral analysis.

5.3.2 Morphological measurements

NI saddlebacks were captured in mist nets (Hen, Cuvier, Tiritiri Matangi and Mokoia Islands) or in roost boxes (Tiritiri Matangi Island) between August 2005 and August 2006. Tarsus length (intra-articular notch at the proximal end of the tarsometatarsus to the middle of the midtarsal articulation; see Taylor and Jamieson (2007) was measured twice to the nearest ± 0.01 mm with vernier callipers. Wing length (maximum unflattened wing chord) was measured to the nearest ± 0.5 mm with a wing rule and weight was measured to the nearest ± 0.5 g with a Pesola spring scale. Sex and age were determined by tarsus length and plumage respectively after Jenkins and Veitch (1991). All measurements were taken by KAP.

SI saddlebacks were captured in mist nets on Ulva Island in April 2009 for subsequent translocation to Orokonui Ecosanctuary (Masuda et al., 2010). Identical measurements were taken but tarsus length was measured three times to the nearest ± 0.1 mm, wing length to the nearest ± 1 mm and weight to the nearest ± 1 g. Sex was determined after Taylor (2007) and age after Heather and Robertson (1996). All measurements were taken by B.M. Masuda.

The NI saddleback tarsus, wing and weight measurements were rounded to the same scale as the SI saddleback data and then all data were examined for normality. We first calculated a resemblance matrix based on Euclidean distance and then checked the NI and SI data for homogeneity of multivariate dispersion (PERMDISP, 9999 permutations) (Anderson, 2006) followed with a non-parametric multivariate analysis of variance (PERMANOVA, 9999 permutations) (Anderson, 2001). We then did a classical discriminant analysis using canonical analysis of principal coordinates (CAP, 9999 permutations) (Anderson and Robinson, 2003; Anderson and Willis, 2003). These analyses were repeated to examine the data by origin (NI or SI) and sex. Finally we examined each variable individually using univariate two-way ANOVA with origin and sex as the grouping variables with Tukey's HSD and Bonferroni-adjusted significance levels.

All vocalisation and morphological multivariate analyses were done using the PRIMER v6 computer program (Clarke and Gorley, 2006) with the PERMANOVA+ add-on package (Anderson et al., 2008). Univariate analyses were done using Statistica v6 (Statsoft, 2002).

5.3.3 Mitochondrial DNA

Blood from five SI Saddlebacks (Big Island) and four NI Saddlebacks (Hen Island) were collected by the authors. Two NI Kokako (*Callaeas cinerea wilsoni*) samples were provided by I. Hogg (University of Waikato) and were collected as part of another study (Hudson et al., 2000). All blood samples were stored in Seutin buffer (Seutin et al., 1991). Five microlitres of blood in buffer was added to 400µl of 5% Chelex solution (BioRad) containing 40µg Proteinase K (Roche) and incubated overnight at 65°C, followed by 10 minutes at 95°C.

Approximately 850bp of control region was amplified using primers L437 (5' CTCACGAGAACCGAGCTACT 3') and H1248 (5' CATCTTCAGTGTCATGCT 3') (Tarr 1995). Each 20µl PCR contained 0.5µM of each primer, 0.8mM dNTPs, 1.5mM MgCl2 and 0.5U of BioTaq (Bioline). PCR amplifications were performed using an Eppendorf Mastercycler ep gradient S and consisted of 94°C for 120 seconds, followed by 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 60 seconds, with a final extension step of 72°C for 240 seconds. Amplified DNA was purified using a High Pure PCR Purification Kit (Roche) and quantified using a Nanodrop ND-1000 spectrophotometer. Templates were sequenced on an ABI 3730 DNA Analyzer using primer L437. Sequences were edited using Sequencher v4.9 (GeneCodes Corporation), aligned with additional sequences from GenBank using Clustal and compared to the NCBI database using BLAST to search for genetic matches (Altschul et al., 1990). Sequences have been submitted to Genbank (Accession numbers XXXXXXXX to XXXXXXXX).

All phylogenetic and distance analyses and distance matrices were performed using PAUP* version 4b10 (Swofford, 2002). Neighbour joining (NJ) trees were constructed using general time-reversible (GTR) corrected distances.

5.4 Results

5.4.1 Song

Highly significant differences in multivariate dispersion of spectral characteristics (PERMDISP, $F = 14.82$, $P = 0.0001$, 9999 permutations) are evident when male specific calls from SI saddleback ($N = 99$ songs from 25 individuals) on Ulva Island are compared with NI

saddleback MRS (N = 2626 songs from 648 individuals; mean number of individuals recorded per island = 45.21 ± 40.65(S.E.); mean number of songs per island = 178.50 ± 157.74; Appendix 1) (Figure 10a). Pairwise comparisons reveal that SI saddleback on Ulva Island show significantly less spectral diversity in song than any NI saddleback population ($P \leq 0.007$).

In contrast chatter calls from SI saddleback (N = 76 calls from 27 individuals) on Ulva Island show significantly greater multivariate dispersion of spectral characteristics than most NI saddleback populations (N = 255 calls from 55 individuals; mean number of individuals recorded per island = 3.93 ± 0.30; mean number of calls per island = 18.21 ± 1.20; Appendix 2) (PERMDISP, F = 5.88, P = 0.0001, 9999 permutations) (Figure 10b). Pairwise comparisons show significant differences ($P \leq 0.007$) to 11 of 14 NI saddleback populations and no difference with NI populations from Cuvier (P = 0.07), Motuihe (P = 0.26) or Whatupuke Islands (P = 0.17).

When examined on a PCA ordination the differences between all NI saddleback populations and SI saddleback from Ulva Island in both song and chatter calls are biologically large (Figure 11). SI saddleback songs from Ulva Island show consistently higher PC1 and PC2 values relative to NI saddleback populations (Figure 11a; Table 7) and consistently higher PC1 values and generally lower PC2 values for chatter calls relative to NI saddleback populations (Figure 11b; Table 7).

All songs from NI populations are significantly different to SI saddleback songs from Ulva Island for measures of log mean pitch (uncorrected P values are shown; $F_{14, 2583}$ = 115.40, P <0.0001) log mean goodness of pitch ($F_{14, 2583}$ = 78.62, P <0.0001), log mean frequency ($F_{14, 2583}$ = 581.00, P <0.0001), log variance in pitch ($F_{14, 2583}$ = 116.32, P <0.0001), variance of frequency modulation ($F_{14, 2583}$ = 53.61, P <0.0001) and variance of mean frequency ($F_{14, 2583}$ = 92.58, P <0.0001) and most populations are significantly different for measures of log duration (post hoc tests showed 10 of 14 NI saddleback populations were significantly different to Ulva; $F_{14, 2583}$ = 44.68, P <0.0001), mean frequency modulation (13/14; $F_{14, 2583}$ = 61.84, P <0.0001), mean entropy (10/14; $F_{14, 2583}$ = 97.13, P <0.0001) and log variance of entropy (13/14; $F_{14, 2583}$ = 46.58, P <0.0001). Only mean amplitude modulation (3/14; $F_{14, 2583}$ = 24.92, P <0.0001) and log variance of goodness of pitch ($F_{14, 2583}$ = 51.58, P <0.0001) showed little differentiation between NI populations and Ulva Island (see Appendix 1 for

mean values for male specific songs from each population). The Bonferroni adjusted experiment-wise significance level for conducting 12 simultaneous tests is 0.05/12 = 0.0042.
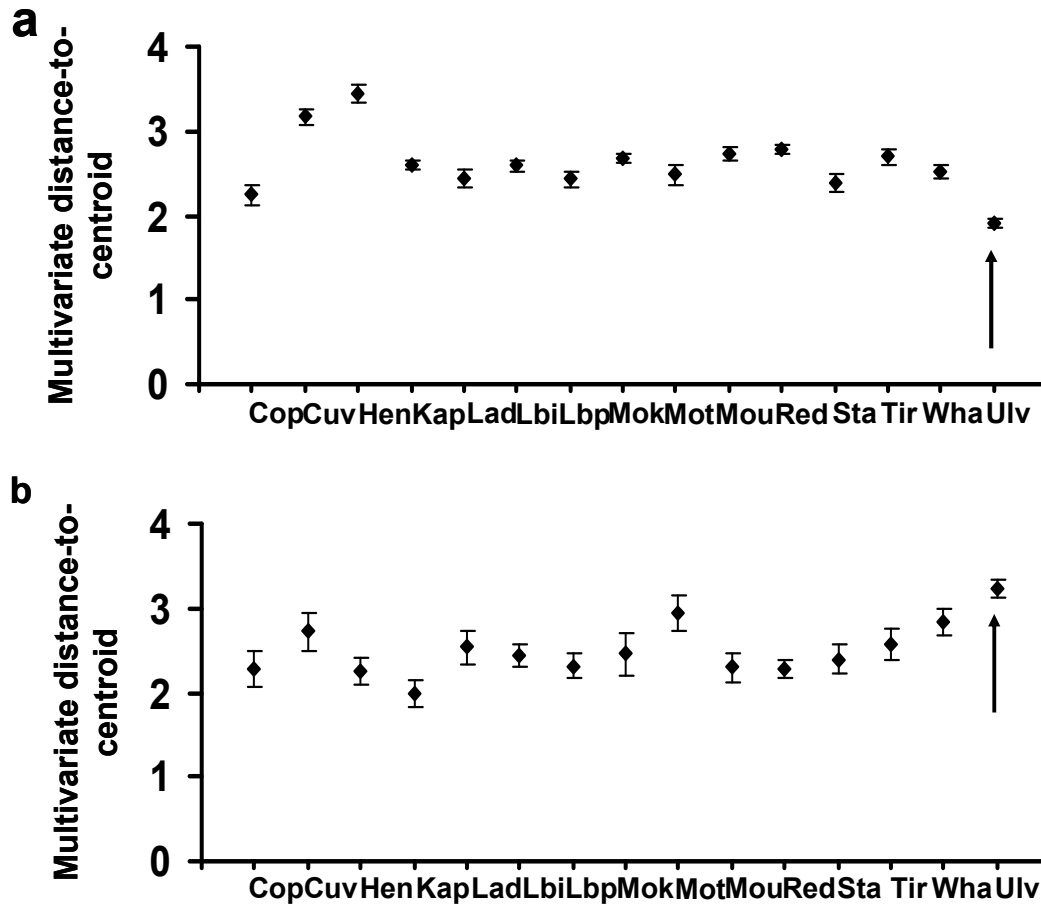
Figure 10. Multivariate distance-to-centroid of spectral variables (average dispersion ± S.E.) of a) NI saddleback MRS and SI saddleback male specific song and b) NI and SI chatter calls within island populations. The SI population (Ulva Island) is indicated by the arrow on each graph. Island abbreviations are; Cop = Coppermine, Cuv = Cuvier, Hen = Hen, Kap = Kapiti, Lad = Lady Alice, Lbi = Little Barrier, Lbp = Pohutakawa Flat Little Barrier, Mok = Mokoia, Mot = Motuihe, Mou = Moutuhora, Red = Red Mercury, Sta = Stanley, Tir = Tiritiri Matangi, Wha = Whatupuke, Ulv = Ulva.

All chatter calls from NI populations are significantly different to SI saddleback songs from Ulva Island for measures of log duration (uncorrected P values are shown; $F_{14, 316} = 152.05$, $P <0.0001$), log mean pitch ($F_{14, 316} = 11.32$, $P <0.0001$), mean frequency modulation ($F_{14, 316} = 30.68$, $P <0.0001$), mean entropy ($F_{14, 316} = 34.17$, $P <0.0001$) mean goodness of pitch ($F_{14, 316} = 22.00$, $P <0.0001$) and log variance of goodness of pitch ($F_{14, 316} = 12.19$, $P <0.0001$). There were fewer differences in measures of mean frequency (post hoc tests showed 8 of 14

NI saddleback populations were significantly different to Ulva; $F_{14, 316} = 6.62$, $P <0.0001$), log variance in pitch (6/14; $F_{14, 316} = 5.15$, $P <0.0001$), variance of frequency modulation (3/14; $F_{14, 316} = 3.58$, $P <0.0001$), variance of entropy (2/14; $F_{14, 316} = 3.75$, $P <0.0001$) and variance of mean frequency (6/14; $F_{14, 316} = 12.00$, $P <0.0001$). There were no significant differences between any NI saddleback population and Ulva Island for mean amplitude modulation ($F_{14, 316} = 12.00$, $P <0.00001$) (see Appendix 2 for mean values for chatter songs from each population). The Bonferroni adjusted experiment-wise significance level for conducting 12 simultaneous tests is $0.05/12 = 0.0042$.

Figure 11. PCA ordination of the average normalised spectral variables for a) NI saddleback MRS and SI saddleback male specific song and b) NI and SI saddleback chatter calls. The NI saddleback populations are indicated by the black triangles and the SI saddleback population (Ulva Island) by the inverted white triangle.

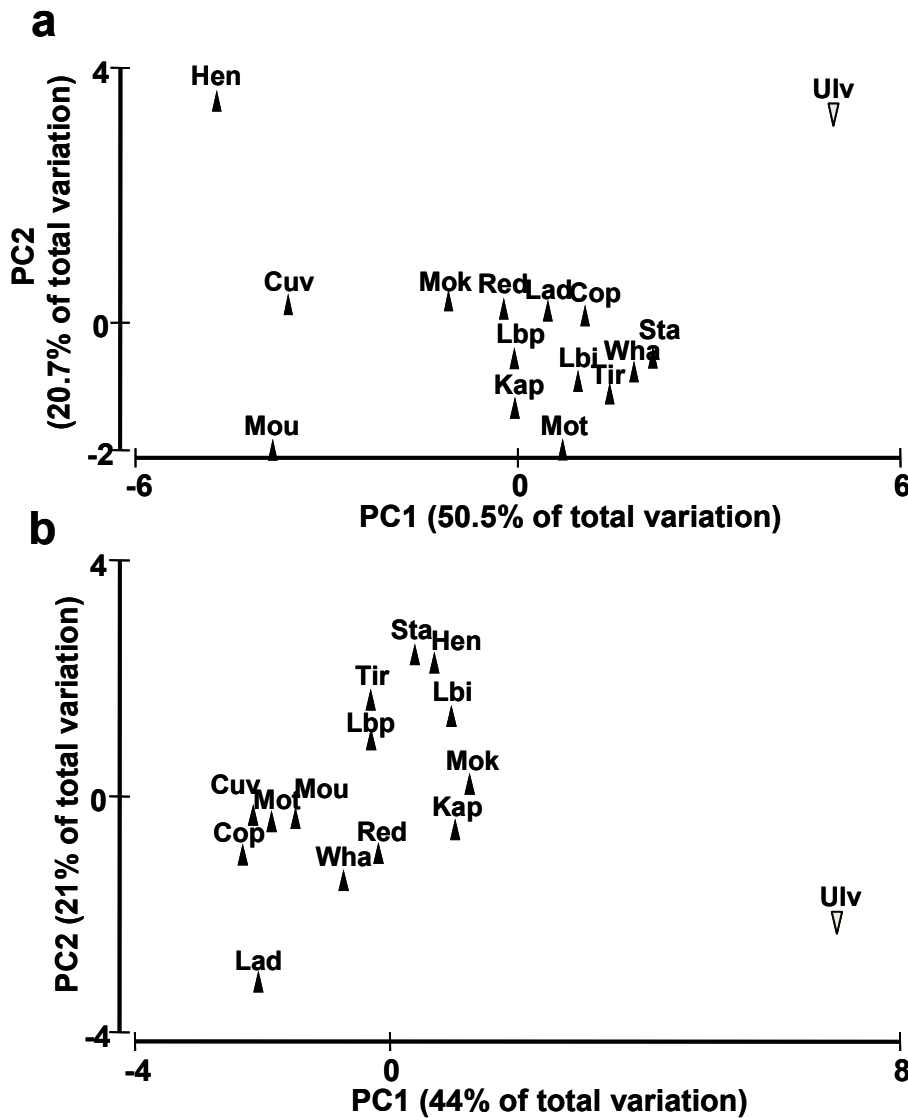**Table 7.** Principal component factor loadings for the analysis of spectral variables from island populations of NI and SI saddleback.

MRS and SI Male specific song			Chatter calls		
Spectral variable	PC1	PC2	Spectral variable	PC1	PC2
Log duration	-0.21	0.38	Log duration	0.38	-0.17
Log mean pitch	0.30	0.40	Log mean pitch	0.31	-0.02
Mean Frequency Modulation	0.33	0.03	Mean Frequency Modulation	-0.28	0.42
Mean Amplitude Modulation	0.15	-0.15	Mean Amplitude Modulation	0.15	0.22
Mean entropy	0.28	-0.35	Mean entropy	-0.21	0.48
Log mean pitch goodness of fit	0.05	-0.57	Mean pitch goodness of fit	-0.40	-0.11
Log mean frequency	0.38	-0.09	Mean frequency	-0.29	0.19
Log variance in pitch	0.37	0.25	Log variance in pitch	0.35	0.28
Variance in Frequency Modulation	0.35	0.03	Variance in Frequency Modulation	-0.25	-0.25
Log variance in entropy	0.32	0.10	Variance in entropy	-0.01	-0.36
Log variance in pitch goodness of fit	-0.03	-0.37	Log variance in pitch goodness of fit	-0.40	-0.06
Variance in mean frequency	0.38	-0.03	Variance in mean frequency	-0.16	0.45

5.4.2 Morphology

There was no significant difference in multivariate dispersion of the morphological variables (PERMDISP, $F = 0.51$, $P = 0.48$; NI saddleback average dispersion = 8.18 ± 0.60 (standard error), $N = 55$; SI saddleback = 7.48 ± 0.59, $N = 40$) but there was a highly significant difference in multivariate location between NI and SI saddleback (PERMANOVA, pseudo-$F_{1, 93} = 26.25$, $P = 0.0001$). A single canonical axis ($\delta 12 = 0.56$) was calculated in the discriminant analysis (CAP) as there are only two groups. The leave one out allocation success was high with 90.52% (86/95) of samples correctly assigned to the right group. NI saddlebacks (92.73%, 51/55) were correctly assigned at a slightly higher proportion than SI saddlebacks (87.50%, 35/40). In permutation tests the trace statistic (0.56) and the first squared canonical correlation (0.56) were highly significant ($P = 0.0001$) (the two statistics are identical because there are only two groups in the CAP) (Anderson et al., 2008).
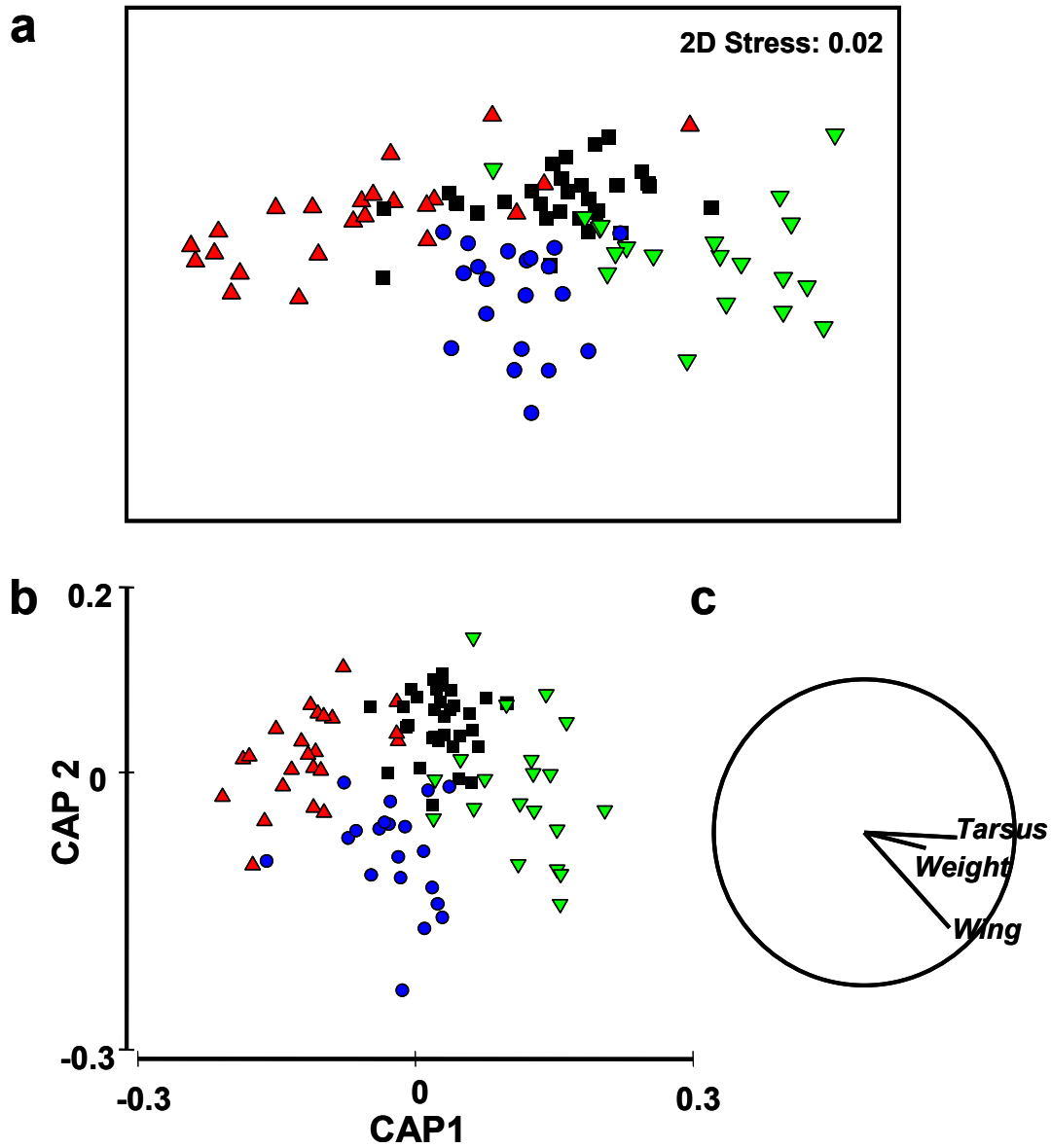
Figure 12. a) An unconstrained non-metric MDS ordination of multivariate differences in morphological measurements for NI and SI male and female saddlebacks. b) A constrained ordination (CAP) of multivariate differences in morphological measurements for NI and SI male and female saddlebacks. c) Correlations of the original variables with the two CAP axes. Red triangle = female NI saddleback, black square = male NI saddleback, blue circle = female SI saddleback and the inverted green triangle = male SI saddleback.

There was a significant difference in multivariate dispersion when the data was examined by origin and sex (PERMDISP, $F = 3.79$, $P = 0.01$) with female NI (6.88 ± 1.02, $N = 23$) and male SI saddleback (6.21 ± 0.71, $N = 20$) showing greater multivariate dispersion than male NI (4.32 ± 0.52, $N = 32$) and female SI saddleback (4.15 ± 0.42, $N = 20$). PERMANOVA is

sensitive to differences in dispersion among groups but Anderson et al (2008) suggest that it is robust to some heterogeneity. Furthermore, examination of the data in an unconstrained non-metric multi-dimensional scaling (MDS) ordination (Figure 12a) suggests that there are differences in location as well as dispersion. Therefore, we conducted a further test of multivariate location by sex and origin which revealed a significant difference between groups (PERMANOVA, pseudo-$F_{3, 91}$ = 45.21, P = 0.0001). The first ($\delta12$ = 0.77) and second canonical ($\delta12$ = 0.51) axes in the discriminant analysis (CAP) clearly separate the data by sex and origin (Figure 12b) and the leave one out allocation success was reasonably high with 84.21% (80/95) of samples correctly assigned to the right group. NI males (90.63%, 29/32) and NI females (86.96%, 20/23) were correctly assigned at a higher proportion than male SI (75%, 15/20) and female SI birds (80%, 16/20). In permutation tests the trace statistic (1.28) and the first squared canonical correlation (0.77) were highly significant (P = 0.0001).

In univariate analyses there are significant differences (uncorrected P values are shown) in tarsus ($F_{1, 91}$ = 51.92, P <0.00001) between NI and SI birds with Tukey's HSD showing significant differences between male NI, female NI, male SI and female SI birds (P < 0.0002) (Figure 13a). There are also significant differences in weight ($F_{1, 91}$ = 32.18, P <0.00001) with Tukey's HSD showing significant difference between all four groups ($P \leq 0.0008$) (Figure 13b) and in wing ($F_{1, 91}$ = 149.77, P <0.00001) with Tukey's HSD showing significant differences between all groups (P < 0.0002) except male and female SI birds (P = 0.07) (Figure 13c). The Bonferroni adjusted experiment-wise significance level for conducting 3 simultaneous tests is 0.05/3 = 0.0167.

5.4.3 mtDNA

774bp of sequence was obtained for NI saddleback, 772 bp for SI Island saddleback and 802bp for NI kokako, resulting in an alignment of 806bp. BLAST searches confirmed the sequences to be control region with closest matches to NI and SI kokako (*C. c. cinerea*). There was no within subspecies variation for any of the taxa. Sequences from the present study were aligned with North and South Island kokako (AF433175 to AF433204) and huia (*Heteralocha acutirostris*, AF433205). As the genbank sequences were shorter than those in the present study, the first 422 – 424bp were excluded from the analyses.

The NJ tree (Figure 14) shows that the NI and SI subspecies of saddleback are sister taxa clearly differentiated from each other and from kokako. A distance matrix of unique haplotypes (Table 8) shows 5.6% divergence between the two saddleback subspecies, which equates to 18 base differences. This is comparable to the 5.0% average divergence (17 – 18 bases) between NI and SI kokako. The level of divergence between saddlebacks and NI kokako is between 15.4% and 19.3% (47 – 57 bases). These data contrast to relatively low levels of divergence (0.6%) within isolated populations of North Island kokako (Murphy et al., 2006).
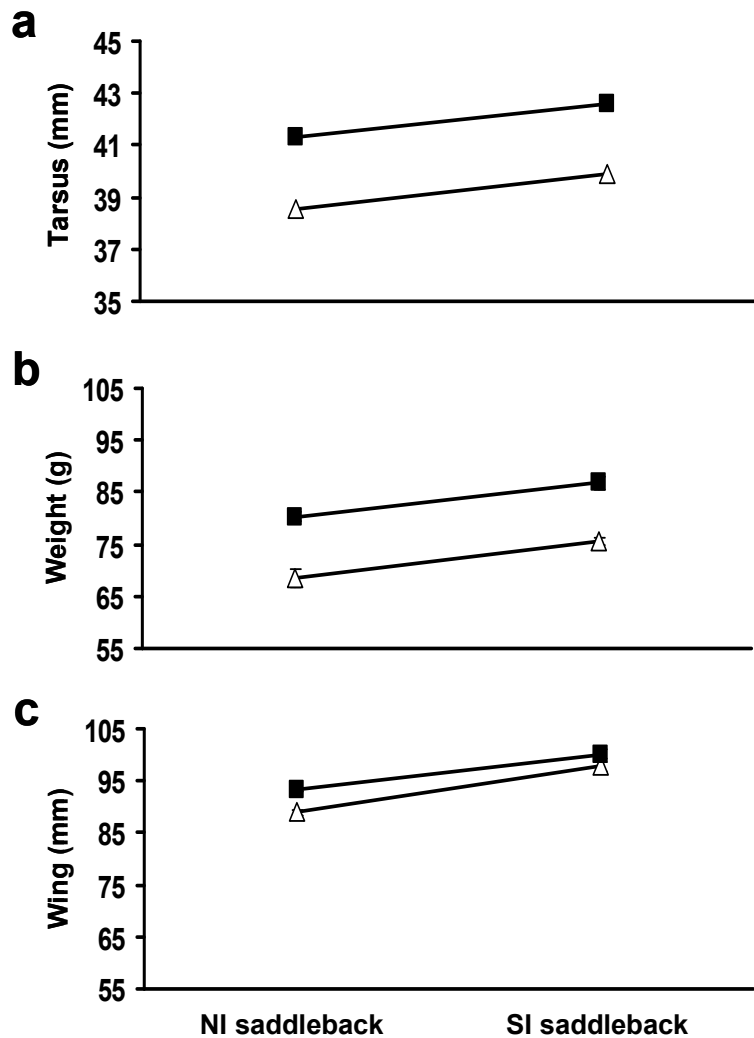
Figure 13. Mean ± S.E. for NI and SI saddleback a) tarsus, b) weight and c) wing. Males are indicated by the black squares and females by the white triangles.
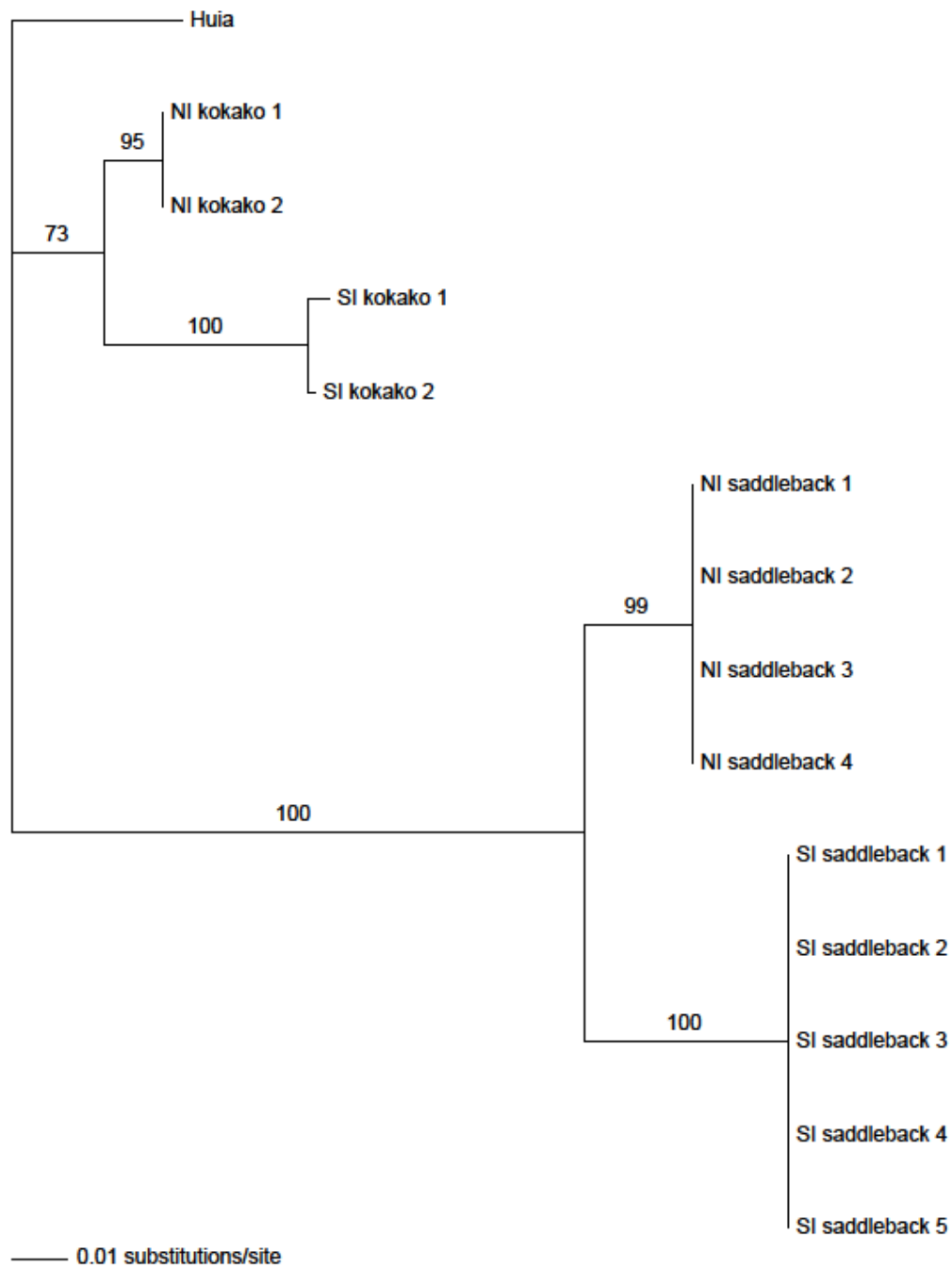
Figure 14. The neighbour joining phylogram is shown with a basal polytomy, which is consistent with previous studies (Shepherd and Lambert, 2007) that were unable to resolve the relationships between these taxa. Bootstrap values >50% are shown.

Table 8. Within and between species/subspecies sequence divergence. The absolute number of nucleotide differences are shown above the diagonal and percentage sequence (general time-reversible) below.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Huia*		13	13	30	31	39	39	39	39	43	43	43	43	43
2. NI kokako 1 (1)	4.2		0	17	18	47	47	47	47	50	50	50	50	50
3. NI kokako 2 (2)	4.2	0.0		17	18	47	47	47	47	50	50	50	50	50
4. SI kokako 4*	10.1	4.8	4.8		2	54	54	54	54	57	57	57	57	57
5. SI kokako 3*	10.4	5.1	5.1	0.5		53	53	53	53	56	56	56	56	56
6. NI saddleback 1	15.0	15.4	15.4	18.2	17.8		0	0	0	18	18	18	18	18
7. NI saddleback 2	15.0	15.4	15.4	18.2	17.8	0.0		0	0	18	18	18	18	18
8. NI saddleback 3	15.0	15.4	15.4	18.2	17.8	0.0	0.0		0	18	18	18	18	18
9. NI saddleback 4	15.0	15.4	15.4	18.2	17.8	0.0	0.0	0.0		18	18	18	18	18
10. SI saddleback 1	17.3	16.6	16.6	19.3	18.8	5.6	5.6	5.6	5.6		0	0	0	0
11. SI saddleback 2	17.3	16.6	16.6	19.3	18.8	5.6	5.6	5.6	5.6	0.0		0	0	0
12. SI saddleback 3	17.3	16.6	16.6	19.3	18.8	5.6	5.6	5.6	5.6	0.0	0.0		0	0
13. SI saddleback 4	17.3	16.6	16.6	19.3	18.8	5.6	5.6	5.6	5.6	0.0	0.0	0.0		0
14. SI saddleback 5	17.3	16.6	16.6	19.3	18.8	5.6	5.6	5.6	5.6	0.0	0.0	0.0	0.0	

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5.5 Discussion

The vocalisation, morphological and mtDNA data presented here, along with plumage (Higgins et al., 2006) and differing vulnerabilities to introduced predators (Hooson and Jamieson, 2003; Lovegrove, 1996), consistently show large and statistically significant differences between NI and SI saddleback populations.

Helbig et al's (2002) criteria demand full diagnosability in at least one of three criteria; 1) individuals of at least one age/sex class can be distinguished from individuals of the same age/sex class of the other taxa by at least one qualitative difference; 2) at least one age/sex class is separated by a complete discontinuity in at least one continuously varying character from the same age/sex class; 3) individuals of at least one age/sex class can be clearly distinguished from individuals of the same age/sex class of all other taxa by a combination of two or three functionally independent characters. The species level separation of NI and SI saddleback clearly fulfil Helbig et al's (2002) first criteria due to completely distinct plumage in both juveniles (which resemble adults in the NI but in the SI have a distinct brown plumage) and adults (yellowish band present in the NI and absent in the SI) (Higgins et al., 2006).

Close examination of the vocalisation characters did not show complete discontinuity thereby Helbig et al's (2002) second criterion is not fulfilled. However, there is virtually no overlap in multivariate dispersion (Figure 10a and b) for two of the three main categories of saddleback vocalisations (the third category, quiet calls, are not included in the analyses presented here), clear differences in multivariate location (Figure 11a and b) and significant differences in almost all mean values (Appendix 1 and 2) when NI island populations are compared to the SI population on Ulva Island. The observed differences are also immediately apparent when listening to NI or SI saddleback vocalisations. In addition, we question whether complete discontinuity in song characters is necessary for assessing differences between taxonomic groups particularly given the very similar vocalisations of pairs of species within many genera, e.g. *Turdus spp*. Further analyses quantifying song characteristics relative to the closely related NI kokako, the only other extant wattlebird, might further elucidate the significance of differences in song between NI and SI saddleback.

Complete discontinuity was not evident in the morphological characters compared, although relatively good discrimination in discriminant analysis (Figure 12b) and significant mean

differences (Figure 13a-5c) were observed. However, we treat these differences with caution as a trend for increasing body size with increasing latitude is apparent in many species (Bergmann, 1847; Blackburn et al., 1999), including most NZ bird species (Higgins et al., 2006). We also note the relatively small sample sizes for morphological data compared here.

The percentage divergence in mtDNA presented here is consistent with NI/SI splits of other bird species such as kokako (Murphy et al., 2006) and blue duck (*Hymenolaimus malacorhynchos*) (Robertson et al., 2007), and is thought to date back about 0.8 - 0.9 million years (Murphy et al., 2006). Divergence times between NI and SI avifauna need to be treated cautiously, but are likely to be within the correct order of magnitude (Baker et al., 1995; Burbidge et al., 2003), and therefore provide ample time for NI and SI saddleback to diverge in vocalisations, morphology and behaviour. The level of sequence divergence (5.6%) between NI and SI form of saddlebacks is sufficient to support elevation to full species status and is similar to that used by Miller and Lambert (2006) to assign species status to the previously subspecific North Island (*Petroica longipes*) and South Island robins (*P. australis*). Hence, we interpret this mtDNA data as evidence of independent evolutionary histories and support for Helbig et al's (2002) second criteria for diagnosability.

Helbig et al's (2002) require an assessment of the likelihood that taxa will retain their genetic and phenotypic integrity into the future to assign a rank of species. In contrast to allopatric taxa, such as the NI and SI saddleback, the reproductive isolation of sympatric and parapatric taxa can be assessed through direct observation. Therefore, the likelihood that the historically allopatric NI and SI forms will remain distinct becomes hypothetical. The role of plumage differences between NI and SI saddleback is unknown although we note that in performing the bow fan warble display (Higgins et al., 2006) male NI birds present their mantle to either their mate or a territorial foe. We also note that SI saddleback can breed whilst in their juvenile plumage. Bird vocalisations play a critical role in species recognition, mate choice and resource defence (Catchpole and Slater, 1995) and likely influence speciation (Grant and Grant, 1997; Slabbekoorn and Smith, 2002). Therefore, playback experiments might also help elucidate the degree of reproductive isolation, particularly as NI saddleback can discriminate between songs that are much more spectrally similar than to the differences observed between NI and SI song (Parker et al., 2010).

Finally, Helbig et al (2002) state allopatric taxa can be assigned species rank if several discrete or continuously varying characters are diagnosable and the sum of character

differences corresponds to or exceeds that seen in closely related sympatric species. This comparison is not possible for saddleback as there are no extant closely related sympatric species aside from the NI kokako.

Saddlebacks are reproductively isolated because of habitat specificity to forest and scrubland habitats and an inability to disperse across gaps greater than 250m (Higgins et al., 2006; Weins, 2004). This, along with human mediated landscape changes means that NI and SI saddleback are extremely unlikely to ever meet in sympatry. Experimental mixing of NI and SI birds would be uninformative, as even distantly related birds can successfully hybridise (Allendorf et al., 2001; Grant and Grant, 1997), and inadvisable given that the two forms of saddleback comprise evolutionary significant units for conservation purposes (Moritz, 1994, 2002). Therefore, we support Holdaway et al's (1989) suggestion that NI and SI saddleback be elevated to full species status.

We acknowledge that existing NI and SI saddleback populations are descendent of relict island populations and both have been through recent (c. 1965) bottlenecks, the SI saddleback bottleneck being particularly severe ($\leq$ 36 birds) (Hooson and Jamieson, 2003; Lovegrove, 1996). These bottleneck events and subsequent conservation management might have influenced the evolution of contemporary populations of saddlebacks. Vocalisation and behavioural data are not available for extinct mainland populations but additional analyses, including genetic and morphometric sampling of museum specimens, would help to confirm these conclusions regarding the taxonomic status of NI and SI saddlebacks.

5.6 Acknowledgements

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Appendix 1. Descriptive statistics (means ± S.E.) for spectral variables measured from NI saddleback MRS and SI saddleback male specific song within each island population. The Bonferroni adjusted experiment-wise significance level for conducting 12 simultaneous test is 0.05/12 = 0.00417.

Island (N songs)	Cop (34)	Cuv (205)	Hen (144)	Kap (272)	Lad (76)	Lbi (248)	Lbp (84)	Mok (295)	Mot (59)	Mou (300)	Red (467)	Sta (78)	Tir (104)	Wha (133)	Ulv (99)	$F_{(14, 2583)}$	P value
Log duration	6.98 (0.07)	7.41 (0.03)	7.27 (0.030)	6.87 (0.02)	6.74 (0.05)	6.59 (0.03)	6.86 (0.05)	6.79 (0.03)	6.52 (0.05)	7.01 (0.03)	6.91 (0.02)	6.81 (0.05)	6.55 (0.05)	6.77 (0.05)	7.06 (0.04)	44.68	<0.0001
Log mean pitch	6.93 (0.04)	6.70 (0.02)	6.86 (0.02)	6.78 (0.01)	6.93 (0.03)	6.79 (0.01)	6.75 (0.03)	6.86 (0.01)	6.84 (0.03)	6.61 (0.01)	6.91 (0.01)	6.94 (0.02)	6.90 (0.02)	6.95 (0.02)	7.51 (0.02)	115.40	<0.0001
Mean Frequency Modulation	22.12 (1.14)	16.23 (0.47)	11.30 (0.56)	14.84 (0.40)	16.73 (0.77)	16.41 (0.42)	16.62 (0.73)	15.21 (0.39)	17.40 (0.87)	15.27 (0.93)	15.15 (0.31)	24.44 (0.76)	20.49 (0.65)	26.82 (0.58)	26.87 (0.67)	61.84	<0.0001
Mean Amplitude Modulation	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.00 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	24.92	<0.0001
Mean entropy	-3.55 (0.11)	-4.29 (0.04)	-5.06 (0.05)	-3.61 (0.04)	-3.85 (0.07)	-3.59 (0.04)	-3.17 (0.07)	-4.14 (0.04)	-3.69 (0.08)	-4.23 (0.04)	-3.71 (0.03)	-2.97 (0.07)	-3.54 (0.06)	-3.95 (0.05)	-3.09 (0.06)	97.13	<0.0001
Log mean pitch goodness of fit	5.47 (0.05)	5.44 (0.02)	4.86 (0.02)	5.63 (0.02)	5.42 (0.03)	5.45 (0.02)	5.40 (0.03)	5.45 (0.02)	5.64 (0.04)	5.70 (0.02)	5.39 (0.01)	5.47 (0.03)	5.51 (0.03)	5.51 (0.02)	5.49 (0.03)	78.62	<0.0001
Log mean frequency	8.21 (0.02)	7.64 (0.01)	7.49 (0.01)	8.14 (0.01)	8.14 (0.02)	8.12 (0.01)	8.08 (0.02)	8.07 (0.01)	8.14 (0.02)	7.58 (0.01)	8.15 (0.01)	8.23 (0.02)	8.21 (0.01)	8.13 (0.01)	8.34 (0.01)	581.00	<0.0001
Log variance in pitch	13.43 (0.15)	12.21 (0.06)	12.41 (0.07)	12.85 (0.05)	13.22 (0.10)	13.03 (0.05)	13.11 (0.09)	13.17 (0.05)	13.01 (0.11)	11.80 (0.05)	13.21 (0.04)	13.61 (0.10)	13.36 (0.08)	13.46 (0.07)	15.13 (0.09)	116.32	<0.0001
Variance in Frequency Modulation	469.35 (23.12)	375.81 (9.41)	265.73 (11.23)	351.44 (8.17)	401.71 (15.46)	411.44 (8.56)	378.04 (14.71)	350.94 (7.85)	432.20 (17.55)	373.84 (7.78)	342.49 (6.24)	467.21 (15.26)	460.69 (13.22)	534.21 (11.69)	618.93 (13.55)	53.61	<0.0001
Log variance in	-0.16	-0.43	-0.43	-0.11	-0.13	0.13	-0.29	-0.36	-0.14	-0.46	-0.29	-0.20	-0.23	-0.30	0.25	46.58	<0.0001

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	Cop	Cuv	Hen	Kap	Lad	Lbi	Lbp	Mok	Mot	Mou	Red	Sta	Tir	Wha	Ulv	F$_{(14, 316)}$	P Value
entropy	(0.07)	(0.03)	(0.03)	(0.02)	(0.04)	(0.02)	(0.04)	(0.02)	(0.05)	(0.02)	(0.02)	(0.04)	(0.04)	(0.04)	(0.04)		
Log variance in pitch goodness of fit	9.33 (0.13)	9.71 (0.05)	8.65 (0.06)	9.38 (0.05)	9.14 (0.09)	9.30 (0.05)	9.04 (0.08)	9.13 (0.04)	9.84 (0.11)	10.22 (0.04)	9.04 (0.04)	9.21 (0.09)	9.34 (0.74)	9.49 (0.07)	9.32 (0.08)	51.58	<0.0001
Variance in mean frequency	12.10 (0.23)	10.53 (0.09)	9.90 (0.11)	12.39 (0.08)	12.65 (0.15)	12.34 (0.08)	12.33 (0.14)	12.14 (0.08)	12.35 (0.17)	10.36 (0.08)	12.28 (0.06)	12.56 (0.15)	12.17 (0.13)	11.94 (0.11)	13.61 (0.13)	92.58	<0.0001

Appendix 2. Descriptive statistics (means ± S.E.) for spectral variables measured from NI and SI saddleback chatter calls within each island population. The Bonferroni adjusted experiment-wise significance level for conducting 12 simultaneous test is 0.05/12 = 0.00417.

Island (N chatter calls)	Cop (17)	Cuv (13)	Hen (20)	Kap (19)	Lad (17)	Lbi (15)	Lbp (26)	Mok (23)	Mot (16)	Mou (18)	Red (27)	Sta (15)	Tir (17)	Wha (12)	Ulv (76)	F$_{(14, 316)}$	P Value
Log duration	5.52 (0.03)	5.66 (0.03)	5.69 (0.03)	5.92 (0.03)	5.63 (0.03)	5.64 (0.03)	5.84 (0.02)	5.74 (0.02)	5.77 (0.03)	5.63 (0.03)	5.73 (0.02)	5.67 (0.03)	5.69 (0.03)	5.70 (0.03)	6.39 (0.01)	152.05	<0.0001
Log mean pitch	7.01 (0.03)	6.95 (0.03)	6.98 (0.02)	6.99 (0.02)	6.89 (0.03)	6.96 (0.03)	6.92 (0.02)	6.98 (0.02)	6.91 (0.03)	6.99 (0.02)	6.96 (0.02)	6.94 (0.03)	7.00 (0.03)	6.91 (0.03)	7.11 (0.01)	11.32	<0.0001
Mean Frequency Modulation	32.08 (1.11)	33.31 (1.27)	32.41 (1.02)	28.96 (1.05)	25.82 (1.11)	31.92 (1.18)	31.45 (0.90)	31.39 (0.95)	28.50 (1.14)	32.77 (1.08)	30.07 (0.88)	31.69 (1.18)	33.52 (1.11)	28.57 (1.32)	19.28 (0.52)	30.68	<0.0001
Mean Amplitude Modulation	0.00 (0.00)	0.00 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	4.09	<0.00001
Mean entropy	-2.89 (0.10)	-2.49 (0.12)	-2.13 (0.10)	-2.44 (0.11)	-3.11 (0.10)	-2.55 (0.11)	-2.36 (0.08)	-3.08 (0.09)	-2.33 (0.11)	-2.55 (0.10)	-2.68 (0.08)	-2.15 (0.11)	-2.31 (0.10)	-2.70 (0.05)	-3.61 (0.12)	34.17	<0.0001
Mean pitch goodness of fit	368.12 (13.41)	371.22 (15.33)	303.76 (12.36)	316.33 (12.68)	364.84 (13.41)	285.70 (14.27)	301.39 (10.84)	282.63 (11.53)	344.42 (13.82)	374.17 (13.03)	311.91 (10.64)	288.17 (14.27)	337.21 (13.41)	318.12 (15.96)	215.24 (6.34)	22.00	<0.0001

Measure																F	p
Mean frequency	3707.29 (54.11)	3710.92 (61.88)	3590.50 (49.89)	3481.90 (51.19)	3659.00 (54.11)	3604.67 (57.61)	3759.92 (43.76)	3647.26 (46.52)	3851.06 (55.78)	3677.56 (52.59)	3669.89 (42.94)	3794.93 (57.61)	3766.59 (54.11)	3681.08 (64.41)	3472.17 (25.59)	6.62	<0.00001
Log variance in pitch	12.65 (0.13)	12.74 (0.15)	13.12 (0.12)	12.95 (0.13)	12.62 (0.13)	13.24 (0.14)	12.98 (0.11)	13.22 (0.11)	12.82 (0.14)	13.00 (0.13)	13.05 (0.10)	13.21 (0.14)	13.01 (0.13)	12.71 (0.16)	13.42 (0.06)	5.15	<0.00001
Variance in Frequency Modulation	566.35 (21.61)	510.08 (24.71)	494.05 (19.93)	540.21 (20.44)	546.53 (21.61)	538.27 (23.01)	510.38 (17.48)	523.83 (18.58)	576.56 (22.28)	536.44 (21.00)	547.07 (17.15)	465.87 (23.01)	486.65 (21.61)	505.08 (25.72)	469.32 (10.22)	3.58	0.00002
Variance in entropy	0.65 (0.06)	0.72 (0.07)	0.66 (0.06)	0.82 (0.06)	0.84 (0.06)	0.71 (0.07)	0.59 (0.05)	0.61 (0.05)	0.88 (0.06)	0.89 (0.06)	0.91 (0.05)	0.66 (0.07)	0.78 (0.06)	0.84 (0.07)	0.82 (0.03)	3.75	0.00001
Log variance in pitch goodness of fit	10.75 (0.16)	10.62 (0.18)	10.33 (0.15)	10.26 (0.15)	10.72 (0.16)	10.06 (0.17)	10.21 (0.13)	10.05 (0.14)	10.52 (0.16)	10.85 (0.15)	10.33 (0.13)	10.08 (0.17)	10.46 (0.16)	10.24 (0.19)	9.40 (0.07)	12.19	<0.0001
Variance in mean frequency	10.94 (0.24)	10.77 (0.28)	12.34 (0.22)	11.36 (0.23)	9.46 (0.24)	12.06 (0.26)	11.00 (0.20)	10.38 (0.21)	11.75 (0.25)	10.89 (0.24)	10.56 (0.19)	11.89 (0.26)	11.71 (0.24)	10.19 (0.29)	11.67 (0.11)	12.00	<0.0001

6 Conclusions and speculations on the future role of song in the divergence of North Island saddleback (*Philesturnus rufusater*) populations

But to discuss whether they are rightly called species or varieties, before any definition of those terms has been generally accepted, is vainly to beat the air.

-Charles Darwin, 1859, On the Origin of Species by Means of Natural Selection

Winker et al. (2007) begin a discussion of species concept debates with the above quote by Darwin (1859). They go on to say that these debates have been a persistent theme in biology, and are likely to remain so, but such debates contribute understanding and need not impede science or conservation. The implicit point is that as scientists we must remain attentive to the mechanisms that might contribute to speciation and receptive to debating the merits of these mechanisms.

The divergence of phenotypic characters within isolated or partially isolated populations facilitates the development of behavioural barriers to gene flow (Darwin, 1871 ; Foster, 1999; West-Eberhard, 1983). There is no question of the critical role of bird song in species recognition, mate choice and resource defence (Catchpole and Slater, 1995) and a logical extension of this role is to one of population divergence and speciation. Considerable geographic variation in song between and within many genera and species of birds is clearly evident (Podos and Warren, 2007). In some species song defines or corroborates species limits (Irwin, 2000; Isler et al., 1998; Mulard et al., 2009; Seddon and Tobias, 2007) whereas in others it provides a recognisable population marker but leaky pre-mating barrier for species (Grant and Grant, 1996) or populations (MacDougall-Shackleton and MacDougall-Shackleton, 2001). Subsequently, there is considerable and ongoing discussion as to the role of bird song in population divergence and speciation (Baptista and Trail, 1992; Fitzpatrick, 1988; Price, 1998, 2008; Remsen, 2005)

The greater body of work examining geographic variation in song has, understandably, focussed on continental species (MacDougall-Shackleton and MacDougall-Shackleton, 2001; Nelson, 1998; Patten et al., 2004). However, as several key studies have demonstrated

(Baker and Jenkins, 1987; Baker, 1994, 1996; Baker et al., 2003; Grant and Grant, 1996) island species provide unique and valuable evolutionary opportunities for studying the role of song. In the work presented in this thesis I have shown that translocated island populations offer a further advantage to studies of both song divergence and other evolutionary processes; assumptions about the origins, size and age of founder populations are unnecessary (Chapter 1; Parker (2008). As such translocated populations offer a beautiful system for studying evolutionary questions. They are free of the artificiality of lab based experiments but avoid the inherent assumptions necessary for many wild studies. Furthermore, in the translocated North Island (NI) saddleback system there is a degree of replication in founder populations (Chapter 1, Figure 1). Given sufficient time the stage is thus set for a demonstration of how population divergence and ultimately speciation might – or might not – occur.

It is clear that song has rapidly (<50 years) changed following translocations of NI saddlebacks and that cultural bottlenecks and post translocation cultural mutation have facilitated these changes (Chapter 2 and 4). Therefore, as with genetic divergence (Templeton, 2008) founder events seem to play a key role in the evolution of song. However, the extent to which these founder events determine the future trajectory of song evolution in NI saddlebacks remains unclear. The observed song diversity might reflect future diversity, i.e. translocation founder events may have caused a relatively fixed and stable song population within each island. Alternatively, ongoing cultural mutations (Jenkins, 1977), along with natural selection, might facilitate continued divergence in song along independent trajectories between island populations to the point where song becomes a definitive population marker (Slabbekoorn and Smith, 2002). It is also plausible that song populations might eventually converge on a common theme as the physiological capability to produce any observed song likely exists in any single male saddleback. This scenario is more likely if a particular song or song type confers fitness advantage for the singer and is subsequently selected for at a disproportionate rate.

Playback experiments (Chapter 4; Parker (2010)) demonstrate, in the short term at least, that NI saddlebacks on Motuihe Island differentiate between familiar and unfamiliar songs. Importantly, they are distinguishing between songs that are acoustically much more similar than those observed in South Island (SI) saddlebacks (*Philesturnus carunculatus*) which have been separated from NI saddlebacks for c. 0.8-0.9 million years (Chapter 5). This, along with the observed differences in established populations of NI saddlebacks (Chapter 2) leads us to

speculate (Chapter 2 and 4) that they currently fit within the early stages of Slabbekoorn and Smith's (2002) model of acoustic divergence (Figure 15, A and B).   In contrast, SI saddleback are at a latter stage of this model (Figure 15, C and D).
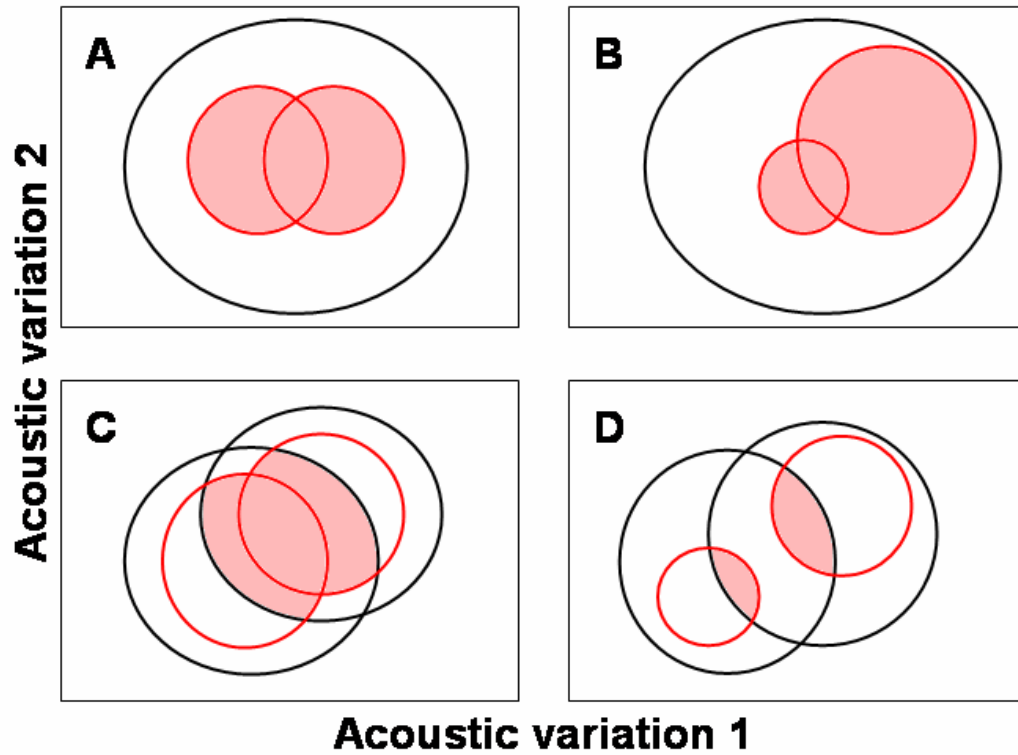

Figure 15. This figure is adapted from Slabbekoorn and Smith's (2002) model of acoustic divergence with song learning. The X and Y axes represent two measures of acoustic variation. The black circles represent the potential (*P*) acoustic variation of a population of individuals while the red circles represent the realised (*R*) acoustic variation of individuals in that population. The overlap of *R* with *P* is shaded. A) Divergence in *R* represents phenotypic variation without any divergence in *P*. B) Divergence of *R* leads to population differentiation but still represents phenotypic variation as it is within the bounds of *P*. C) Divergence of *R* and *P* but the mutual overlap in *R* makes the divergence an incomplete population marker. D) Divergence of *R* and *P* with song becoming a reliable population marker.

Playback experiments are informative but ultimately, as with many of the various species concepts (Helbig et al., 2002; McKitrick and Zink, 1988; Weins, 2004), the significance of allopatric change can only be truly tested in sympatry. This will be the focus of future work with planning currently underway for experimental translocations which, as advocated in Chapter 1, will combine scientific questions with conservation and community outputs.

It is unclear if the currently observed song differences between islands will translate into definitive mating barriers following experimental mixing of allopatric populations in sympatry. I suspect that birds from different islands would probably mix in sympatry. However, there might be assortive mating in the early stages of a new sympatric population descendent from allopatric populations. As with the related kokako (*Callaeas wilsoni*) this might disappear at the new site when the first generation commences breeding. Songs characteristic of some populations might also prove to be more successful than those from others, e.g. the more diverse songs from the ancestral population on Hen or the first level bottleneck populations (Chapter 2), might be preferred over the less diverse songs from third bottleneck populations.

I have demonstrated how population isolation caused by conservation management can have a profound impact on a fundamental aspect of a species biology (Chapter 2 and 4). Ongoing research should be directed at monitoring song diversity within and between island populations of NI saddleback over time; decades, hundreds and even thousands of years. This will ascertain the degree of temporal effects and further elucidate the role of song in population divergence. Translocation might have set the stage for change but only time will reveal the nature of the show.

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Writing a book is an adventure. To begin with, it is a toy and an amusement; then it becomes a mistress, and then it becomes a master, and then a tyrant. The last phase is that just as you are about to be reconciled to your servitude, you kill the monster, and fling him to the public.

-Winston Churchill